Studies on clinical symptoms, diagnosis and treatment in pemphigoid diseases

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List of abbreviations

AIBD	autoimmune blistering diseases
anti-LN-332 MMP	anti-laminin-332 mucous membrane pemphigoid
BMZ	epidermal basement membrane zone
BP	bullous pemphigoid
Coll VII	type VII collagen
CS	corticosteroids
DIF	direct immunofluorescence microscopy
EBA	epidermolysis bullosa acquisita
ELISA	enzyme linked immunosorbent assay
FOAM	fluorescent overlay antigen mapping
G6PD	glucose-6-phosphate dehydrogenase
GFHD	G.F.H. Diercks, MD, PhD
HDs	hemidesmosomes
IIF	indirect immunofluorescence microscopy
Inf	inflammatory phenotype
IP	immunoprecipitation
KO	knock-out immunofluorescence analysis
LAD	linear IgA bullous dermatosis
LN-332	laminin $\alpha 3\beta 3\gamma 2$
LPP	lichen planus pemphigoides
MB	classic mechanobullous phenotype
MFJ	M.F. Jonkman, MD, PhD
MMP	mucous membrane pemphigoid
MO	monkey esophagus
MTX	methotrexate
NC1	non-collagenous amino-terminal globular domain
NC2	34-kDa, non-collagenous carboxy-terminal globular
NCHA	the Netherlands Consortium for Healthy Ageing
OCP	ocular cicatricial pemphigoid
PG	pemphigoid gestationis
sAIBD	subepidermal autoimmune blistering diseases
SSS	salt split skin analysis
TPMT	thiopurine methyltransferase activity
180-kD antigen	BP180, BPAG2, type XVII collagen
230-kD antigen	BP230, BPAG1
290-kDa antigen	type VII collagen, C7

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Introduction

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Introduction

Pemphigoid is a group of subepidermal autoimmune blistering diseases (sAIBD) which comprise many subtypes with a clinical heterogenous appearance, a specific laboratory diagnosis and tailor made treatment. This thesis focuses on the clinical symptoms, diagnosis and treatment in pemphigoid diseases, especially bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), anti-laminin-332 MMP (anti-LN-332 MMP) and epidermolysis bullosa acquisita (EBA)

Epidemiology

Pemphigoid diseases show a rising incidence in the past decades. In Europe the incidence of BP is between 13.4-21.7 per one million people.[1-4] In the UK, Germany and France the incidence of BP has increased substantially (two to five times). This rising incidence may be due to the increasing age of the general population and the use of multiple drugs in elderly. Furthermore more sensitive and specific diagnostic assays are developed.[5] BP occurs preferentially in elderly with median age of 80 years old and is known to have significantly associated increased mortality rates compared to the general population.[4] Cortes et al. reported a 1-year probability of death of 20.9% in BP. The mortality rate was three times higher in the BP cohort compared to the general Swiss population.[6] Associated neurological disorders and patients' general condition are both risk factors and major prognostic factors for BP patients.[7] Specific associations are reported between BP and Parkinson's disease, dementia, stroke, and multiple sclerosis.[8] The identification and characterization of genes and pathways that contribute to the presence or absence of BP and neurological diseases is one of the themes to be studied. The Netherlands Consortium for Healthy Ageing (NCHA) is an alliance between the Groningen University Medical Centre, the Leiden University Medical Centre, the Erasmus Medical Centre, the Academic Medical Centre, the VU University and business partners Unilever, Philips, Galápagos, McRoberts, Pfizer and DSM. The NCHA integrates scientific disciplines, technological innovations and biomedical research in the largest collection of world-renowned human cohort studies. Pemphigoid diseases, especially BP, is because of the association with age and neurological disease a disease of elderly with many comorbidities. The NCHA can be of use to learn from patient's genetics and metabolic constitution, life style and the interaction with environmental factors like socio-economic status, and use of multiple drugs to identify the factors who contribute to the rising incidence of pemphigoid diseases.

Clinical symptoms

Pemphigoid comprises different subtypes like BP, MMP, ocular cicatricial pemphigoid (OCP), anti-LN-332 MMP, anti-p200 pemphigoid, anti-plectin pemphigoid, linear IgA bullous dermatosis (LAD), pemphigoid gestationis (PG), lichen planus pemphigoides (LPP), Brunsting-Perry pemphigoid and epidermolysis bullosa acquisita (EBA).[5,9] The clinical appearance of patients with pemphigoid diseases is various, although some subtypes have specific clinical symptoms (Table 1).

BP is the most common form of pemphigoid. Pruritus, urticaria and tense blisters are the three clinical pillars stated in the "Definitions and outcome measures for bullous pemphigoid".[10] The predilection sites are the flexor surfaces of the arms and legs, axillae, groin, flanks and abdomen (Fig 1-A). Oral lesions are seen in a minority of patients and the lesions are usually transient. BP may start with pruritus in the prodromal stage, while blisters develop weeks or months later. Confusing is the subset of patients with immunopathological findings of BP, pruritus, but no blister development for years (Fig 1-B). In a Swiss study 20% of 160 diagnosed BP patients presented without blisters.[11] In the literature there is no consensus on how to name this subset of patients. The coined terms include 'pruritic pemphigoid', 'pemphigoid nodularis', 'papular pemphigoid', 'prurigo-nodularis like pemphigoid', 'non-bullous BP', 'prodromal BP', 'cutaneous pemphigoid' and 'BP incipiens'.[12-19] It is important to be aware of this subtype of pemphigoid because these patients, mainly elderly, presenting with pruritus sine materia (no skin lesions) or wit non-bullous skin lesions are frequently misdiagnosed as xerosis, drug reaction, dermatitis, renal impairment, liver impairment or scabies.

MMP predominantly affects different types of mucosa and sometimes the skin.[20] Compared to BP, clinical symptoms appear earlier in life (mean age 60-65 years). The oral cavity (85%) is the most common site involved, followed by ocular disease (65%). The nasal (20-40%), pharyngeal (20%), laryngeal (5-10%), anogenital (20%) and esophagus (5-15%) region can also be affected.[9,20,21] All sites involved tend to heal with scar formation, although in the oral cavity re-epithelisation without scarring can occur. OCP is a subtype of MMP in which the eyes are the only affected mucosa, causing progressive cicatrization. OCP usually starts unilaterally with clinical features of dry eye, conjunctivitis, trichiasis, symblepharon formation and finally resulting in blindness when not treated accurate. Patients with OCP are scored by the Tauber classification system.[22]

In patients with anti-LN-332 MMP, airway obstruction due to pharyngeal and laryngeal involvement is a serious complication and is seen more often than in other forms of MMP.[23] In the first instance patients present with aphonia (loss of voice) due to oedema, erosions and ulcerations of the supraglottic area. This is followed by scarring of the larynx, and acute upper airway obstruction due to initial laryngeal oedema may occur, necessitating tracheotomy.[24,25] The nose, esophagus and anogenital region are other frequent affected mucosal surfaces.[26] Patients with anti-LN-332 MMP have an increased relative risk for malignancy and should be thoroughly screened. Adenocarcinoma like lung cancer and stomach cancer were found mostly.[27-29]
Anti-p200 pemphigoid is a rare disease and clinical heterogenous. Patients present with tense blisters and urticaria, mimicking BP. Generally the disease starts at a younger age compared to BP. Till now, the classical clinical presentation and pathogenic autoantibodies in this disease is not clarified.[30,31]
Clinically EBA can present at any age with either the classic mechanobullous (MB) phenotype or as the inflammatory (Inf) phenotype that mimicks other pemphigoid diseases as BP, MMP or LAD.[32] The classical MB phenotype mimics dystrophic epidermolysis bullosa hereditaria and is a non-inflammatory mechanobullous disease primarily involving trauma-prone areas of skin, such as the hands, elbows, knees, and feet. These lesions heal with scarring resulting in milia. Inf EBA is clinical heterogenous with a widespread of non-scarring vesiculobullous eruption that mainly involves the intertriginous region and flexures, and in some cases the oral mucosa. Different subtypes may occur like: Inf-BP like, Inf-MMP like or Inf-vesicular pemphigoid like. Buijsrogge et al. described the many faces of EBA and concluded that 2/3 of the EBA patients show the Inf phenotype.[33] IgA EBA presents with intense pruritic widespread or localized vesicles with frequently involvement of mucosal surfaces without cicatrization.

	Target antigens	Clinical symptoms	Treatment
Bullous pemphigoid	BP180 BP230	Pruritus, urticaria, tense blisters, eczema, papules or nodules without predominant mucosal involvement	Potent topical CS, systemic CS. Antibiotics, nicotinamide, azathioprine, mycophenolate mofetil, mycophenolic acid, methotrexate, dapsone
Mucous membrane pemphigoid	BP180 BP230	Erosions and blisters of the oral, nasal, eyes, pharyngeal, laryngeal, esophagus and anogenital mucosa	Low risk: Potent topical CS, antibiotics, dapsone High risk: systemic CS, dapsone, cyclophosphamide, mycophenolate mofetil, mycophenolic acid
Anti-laminin 332 mucous membrane pemphigoid	LN-332	Erosions and ulcerations of the supraglottic area, nose, eyes, esophagus and anogenital region, aphonia, dyspnoe	Systemic CS, dapsone, cyclophosphamide, azathioprine, mycophenolate mofetil, mycophenolic acid
Ocular cicatricial pemphigoid	BP180	Dry eyes, conjunctivitis, trichiasis, symblepharon, blindness (Tauber classification system)	Systemic CS, dapsone, cyclophosphamide, azathioprine, mycophenolate mofetil, mycophenolic acid
Linear IgA bullous dermatosis	BP180 LAD-1	Tense blisters and erosions ("crown of jewels" and "string of pearls"). Oral, nasal and genital erosions and crustae (70%)	Potent topical CS, systemic CS, dapsone, sulfapyridine
Epidermolysis bullosa acquisita, MB phenotype	Collagen VII	Blisters and erosions of the trauma-prone areas of the skin, healing with milia and scarring	Systemic CS, colchicine, azathioprine, mycophenolate mofetil, mycophenolic acid
Epidermolysis bullosa acquisita, Inf phenotype	Collagen VII	Non-scarring pruritic vesiculobullous eruption, without predominant mucosal involvement	Potent topical CS, systemic CS. Antibiotics, nicotinamide, azathioprine, mycophenolate mofetil, mycophenolic acid, methotrexate, dapsone
Pemphigoid gestationis	BP180	Intense pruritic urticarial rash, papules and tense blisters starting around umbilicus and then spread over the body	Potent topical CS, cetirizine
Brunsting-Perry pemphigoid	BP180 BP230	Erosions and blisters limited to the head, face, neck and upper trunk leaving atrophic scars	Potent topical CS or systemic CS. Azathioprine, mycophenolate mofetil, mycophenolic acid
Anti-p200 pemphigoid	P200	Tense blisters and urticaria	Treatment algorithm for BP
Lichen planus pemphigoides	BP180 BP230	Tense blisters independent of the lichenoid plaques and papules of lichen planus	Treatment of lichen planus and treatment algorithm for bullous pemphigoid

Table 1: Target antigens, clinical symptoms and treatment of pemphigoid diseases. CS: corticosteroids; MB phenotype: mechanobullous phenotype; Inf phenotype: inflammatory phenotype

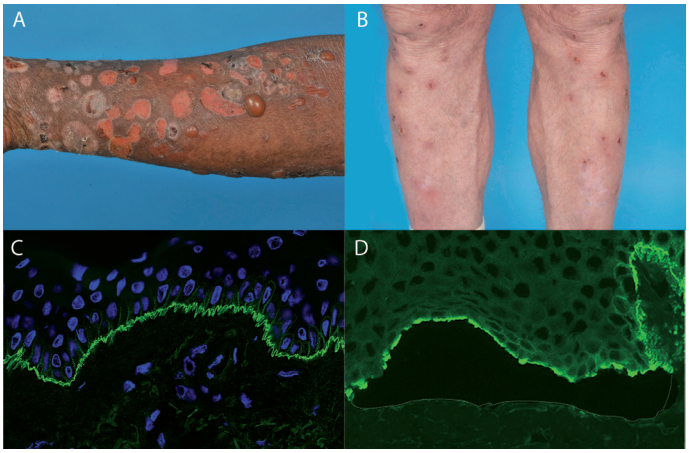

Figure 1: Clinical and immunopathological features of bullous pemphigoid. Tense blisters and erosions on the left arm (A); Excoriated papules and nodules on both legs (B); Direct immunofluorescence microscopy perilesional showing deposits of IgG in the n-serrated pattern (C); Indirect immunofluorescence microscopy on salt split skin analysis showing epidermal binding of the blister (D)

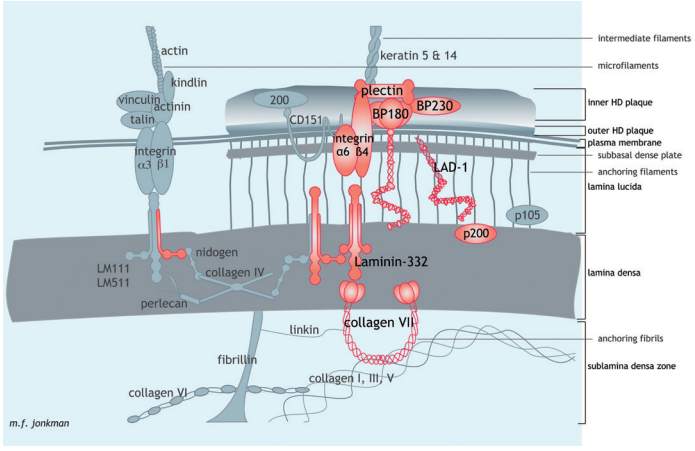

Figure 2: Overview of the epidermal basement membrane zone. The proteins that are targeted by autoantibodies in pemphigoid diseases are colored in red.

Target Antigens

Pemphigoid is characterized by subepidermal cleavage of the skin due to circulating autoantibodies targeting antigens in the epidermal basement membrane zone (BMZ) (Fig 2).[9] The basal keratinocytes in the skin are attached to their underlying BMZ via specialized adhesion complexes termed hemidesmosomes (HDs). HDs are multiprotein complexes and contribute to the stability of stratified epithelia of the skin, gastro-intestinal and respiratory tract. Defects in components of these adhesion complexes often result in tissue fragility and blistering of the skin.[34-36]

The 180-kD antigen (BP180, BPAG2, or type XVII collagen), and the 230-kD antigen (BP230, BPAG1) are the main target antigens in BP, MMP, LAD, PG, OCP, Brunsting-Perry pemphigoid and LPP.[5,9] BP180 is a 180-kDa transmembrane glycoprotein that ultrastructurally spans the lamina lucida and curves back from the lamina densa into the lamina lucida.[37-39] The non-collagenous NC16A ectodomain of BP180 is the immunodominant region in BP.[40] BP230 is a cytoplasmic protein involved in the anchorage of intermediate filaments (IF) to the cytoskeleton.[37,41]

Anti-LN-332 MMP is a form of pemphigoid with circulating autoantibodies targeting LN-332. This is a heterotrimeric protein consisting of $\alpha 3$, $\beta 3$ and $\gamma 2$ subunits (laminin $\alpha 3\beta 3\gamma 2$). LN-332 is present in the basal lamina of various epithelia including stratified squamous epithelium, and connects hemidesmosomes to anchoring fibrils by interlinking integrin alpha6beta4 and BP180 to type VII collagen.[42-45] The 290-kDa antigen (type VII collagen, C7), the major structural component of the anchoring fibrils located in the BMZ, is the targeted antigen in EBA.[46,47] The molecule itself is a trimer consisting of three identical alpha-chains. Each chain contains a 145-kDa central collagenous triple-helical region, that is flanked by a large 145-kDa, non-collagenous amino-terminal globular domain (NC1) and a smaller, 34-kDa, non-collagenous carboxy-terminal globular domain (NC2). Within the extracellular space these molecules form anti-parallel tail to tail dimers which then conglomerate on the lateral side to form anchoring fibrils.[48,49] The immunodominant epitopes of C7 are particularly located within the NC1 domain and in the minority in the NC2 domain or central collagenous domain.[50-52]

Anti-p200 pemphigoid is characterized by circulating autoantibodies against the 200-kDa-protein of the lower lamina lucida. Dainichi et al. described that the 200-kDa-protein corresponded in the majority of their patients (90%) with the C-terminus of laminin gamma 1, an extracellular matrix glycoprotein composing several forms of laminin heterotrimers, and named the disease anti-laminin $\gamma 1$ pemphigoid.[30] However, the pathogenic relevance of these autoantibodies has not been demonstrated yet.[31]

Diagnosis

Diagnosis in patients clinically suspected for pemphigoid diseases is based on a combination of clinical features, histopathological examination, detection of in vivo bound autoantibodies (IgG, IgA, IgM, C3c and fibrin) in skin or mucosa (DIF), and detection of antigen specific circulating autoantibodies in the serum (IIF).

The most important use of a skin biopsy for histopathology is to determine the level of split in the skin. Intra-epidermal split is associated with pemphigus and subepidermal split is associated with pemphigoid. The type of infiltrate seen in the biopsie may be a clue for the diagnosis, but no infiltrate is specific for a subtype of pemphigoid. Because multiple diseases are associated with intra- or subepidermal split, immunofluorescence microscopy is mandatory to diagnose pemphigoid.

DIF allows us to demonstrate linear depositions of immunoreactans in the BMZ. These linear depositions of IgG or IgA can have two distinct serration patterns: the n-serration, and the u-serration pattern.[53] Recognition of DIF serration patterns must be standardized for optimal determination: i) perilesional biopsies of non-scarring skin not exposed to topical corticosteroids, ii) transporting biopsies without freezing in saline for 24 hours, iii) thin cryosections (4 µm thickness or less), iv) lens objective of at least 40x and appropriate microscope filters. The u-serration pattern confirms the diagnosis EBA, and represents immunoglobulin depositions in upstanding arms ("grass") of the sublamina densa zone between the rootlets of basal keratinocytes. In all other forms of pemphigoid the antigens are located in the lamina lucida or above, so the immunodepositions follow the rootlets of the basal keratinocytes showing the n-serration pattern (Fig 1-C).[5,53] In few cases the serration pattern is undeterminable. This can be due because the serration pattern can not always be recognized, especially in mucosal biopsies. In patients with OCP, DIF (conjunctiva) is frequently the only positive assay. These biopsies can be performed by the dermatologist or ophthalmologist. DIF serration pattern analysis has found limited use nowadays, although the criterion is mentioned in textbooks,[54] and in the forthcoming European guideline on BP. The limited use might be caused by uncertainty and lack of training of the IF microscopists. In our Centre for Blistering Diseases in Groningen, the Netherlands, DIF serration pattern analysis is routinely used. With the use of this assay we are able to make in almost any case suspected for pemphigoid a final diagnosis when DIF is positive. DIF serration pattern analysis can also be used as a diagnostic criterion in EBA. The current criteria to diagnose EBA consists of: (i) acquired bullous disease within the defined clinical spectrum, (ii) histologically subepidermal cleavage, (iii) DIF of perilesional skin revealing linear IgG along the BMZ, (IV) positive alternative laboratory test like IF on salt split skin analysis (SSS), IIF using substrate

deficient in BMZ molecules, Western blotting, Fluorescent Overlay Antigen Mapping (FOAM), and ELISA.[55] When the criterion DIF of perilesional skin revealing linear IgG along the BMZ is used alone, no difference can be made between EBA and other forms of pemphigoid. Especially with the knowledge that in about 50% of the EBA patients no circulating autoantibodies in the serum can be detected. In these cases u-serration pattern by DIF is decisive.

Another subtype of pemphigoid, anti-LN-332 MMP, is diagnosed on the following criteria described previously: (i) chronic subepithelial blistering lesions of mucous membranes and skin, (ii) DIF microscopy showing linear deposits of IgG with or without C3c along the BMZ, (iii) IIF microscopy showing IgG autoantibodies binding to the dermal side salt-split human skin, and (iv) circulating IgG anti-BMZ autoantibodies that immunoprecipate LN-332 from human keratinocyte extracts.[26,56,57] Anti-p200 pemphigoid, EBA and anti-LN-332 MMP all show IgG autoantibodies binding to the dermal side salt-split human skin.

Serological analysis for circulating autoantibodies is of great use in the diagnostic approach. Traditionally IF is performed on frozen sections of standard substrates like monkey esophagus, human split-skin and rat bladder.[58] Serum samples from patients with MMP are serological positive in a low percentage (50-80%) and when positive it is in low titres. Circulating IgA autoantibodies can be detected in the majority of the MMP patients (60%). When IgG and IgA autoantibodies are both present, patients have a more severe and persistent MMP compared to patients with only IgG autoantibodies.[59,60]

SSS is useful to differentiate between different groups of autoantigens. Incubation of human skin in 1.0 M NaCl leads to a reproducible split through the lamina lucida.The hemidesmosomes associated antigens 180-kDa, 230-kDa, plectin, integrin alpha6beta4 and LAD-1 are located in the epidermal side of the blister (Fig 1-D), and the antigens LN-332, p200 and 290-kDa are located at the dermal side of the blister.[61-64] The combination of serration pattern analysis by DIF and SSS is for the clinician in many cases already decisive. These assays should be available in any routine diagnostic laboratory.

In specialized laboratory and in Blistering Centres additional tests like immunoblot, enzyme-linked immunosorbent assay (ELISA), immunoprecipitation (IP) , knock-out immuno-fluorescence analysis (KO) and FOAM are available.

Immunblot (also called Western blot) is an analytical technique that allows to demonstrate specific protein expression. Proteins to be analyzed are resolved by electrophoretic separation over a polyacrylamide slab-gel in the presence of sodium dodecyl sulphate (SDS-PAGE) and brought on a membrane. The interaction between the membrane and patients' serum makes the antigen visible which is targeted by IgG. The advantage of this assay is the possibility to test multiple

antigens in one immunblot. On the other hand conformational epitopes can be destroyed by the denaturation process whereby the IgG binding to the membrane is not feasible.[65]
ELISA is a diagnostic criterion to detect the presence and the titer of specific autoantibodies in the serum of a patient. A specific antigen is coated onto a plastic well and the well is incubated with patients' serum. When IgG to this antigen is present, it will bind the immobilized antigen. Then after washing the well, the amount of IgG is visualized by colorimetric reaction. The intensity of the color is a measure of the amount of autoantibodies in the blood of the patient. In contrast to immunblot, ELISA is a quantitative assay and can be used as a tool to monitor disease activity in different subtypes of pemphigoid.[66-68]
Recently new ELISA's for the detection of autoantibodies against C7 have been developed that has the recombinant NC1 and NC2 coll VII domains coated to the plate. A high sensitivity (>93%) and specificity (>96%) has been reported.[68,69] However all these studies relied on sera that were positive by SSS analysis.

IP is used to precipitate antigens out of an extract using patient autoantibodies immobilized on a solid support, such as agarose or magnetic beads. IP can in contrast to immunoblot detect conformational epitopes. IP is not general used as it requires radiolabeled protein extracts and therefore specially equipped laboratories.
KO is an assay which allows to differentiate between anti-LN-332 MMP and EBA patients. The serum of the patient will be brought on a section of C7-deficient skin from a patient with severe recessive dystrophic epidermolysis bullosa, and on a section of LN-332 deficient skin of a patient with Herlitz-type junctional epidermolysis bullosa. When the serum does not bind to C7 deficient skin, but will bind to LN-332 deficient skin, this confirms the presence of anti-C7 autoantibodies.[70]
FOAM is a specialized technique developed in our laboratory and makes it possible to distinguish between deposits above (BP, anti-LN-332 MMP) and below (EBA) the lamina densa. By double staining the deposited IgG and the antigen overlap or non-overlap can be visualized.[71] FOAM can be very useful as a diagnostic criterion in cases in which DIF, IIF, immunblot and ELISA are not conclusive.
With all these tests available in different laboratories, clinicians must be able to solve the most complicated diagnostic cases of pemphigoid. By combination of these tests in Centres for Blistering Diseases, the knowledge in the diagnostic approach in pemphigoid diseases will be centralized, and more accurate diagnoses can be made.

Treatment

Treatment of pemphigoid is challenging because of the variable age of the patients, differences in disease severity, progressive scarring of mucosa and the presence of comorbidities (Table 1). The aim of the therapy is to induce healing of the lesions on the skin and/or mucous membranes and to minimize adverse effects of the treatment as much as possible.

Topical and systemic corticosteroids (CS) are first line therapy in patients with pemphigoid. Topical CS should be considered in any patient with BP.[72] The French "Group Bulle" showed the efficacy of topical clobetasol propionate cream (10-30g) application on the whole body, progressively tapered over 4 months in moderate BP.[73] The same group also examined the effectiveness of topical clobetasol propionate cream compared to treatment with oral CS in patients with BP, showing that topical CS is effective and increased survival for both moderate and severe BP and is superior to systemic CS for extensive disease.[74] It is likely that the high efficacy of whole body topical clobetasol propionate application is due to both local and systemic effects. Van Velsen et al. have showed the systemic absorption and effects of whole body application of clobetasol propionate 0.05% cream in patients with atopic dermatitis.[75] Systemic absorption of clobetasol propionate 0.05% cream has never been described before in BP patients.

According to current evidence systemic CS is the best established treatment.[76] The dose of systemic CS given is 0.5-0.75 mg/kg/day. When after four weeks lesions tense to heal or stabilize, treatment can be judged as successful and tapering of dose should be started. Dose above 0.75 mg/kg/day is not more effective and is associated with higher mortality and increased adverse effects.[76] During treatment with systemic CS one should be aware of potential risks such as hypertension, osteoporosis, hyperlipidemia, diabetes mellitus, psychiatric disorders, cataract, glaucoma, and systemic infections which can be life threatening.[76] Gastric protection and osteophorosis prophylaxis must be prescribed.

Adding a CS sparing adjuvant is important to reduce the adverse effects caused by longterm CS use. In BP one can choose for antibiotics (tetracycline, doxycycline, minocycline) with or without nicotinamide (up to 1500 mg), azathioprine (2-3mg/kg/day), mycophenolate mofetil (2g/day), mycophenolic acid (720 mg twice/day), methotrexate (10-20 mg/week) or dapsone (100-200mg/day). Before introducing a CS sparing adjuvans routine blood screening and specific serological assays like thiopurine methyltransferase (TPMT) activity (azathioprine) and glucose-6-phosphate dehydrogenase (G6PD) deficiency (dapsone) must be performed. The use of rituximab (anti-CD20 monoclonal antibody), high-dose intravenous immunoglobulin, plasmapheresis or immunadsorption is described in refractory patients.[77-80]

As stated before treatment of patients with MMP is challenging because of the scarring potential

of certain mucous membranes like ocular and laryngeal mucosa. Patients can be divided in i) low risk MMP (affection oral mucosa with or without skin lesions), ii) high risk MMP (affection of any other mucosa). Low risk MMP can be treated with topical potent CS with or without antibiotics or dapsone. High risk MMP is treated more aggressively with systemic CS and dapsone (100-200mg/day), cyclophosphamide (100-200mg/day), mycophenolate mofetil (2g/day) or mycophenolic acid (720 mg twice/day). Dexamethasone pulse therapy or systemic CS (1.0mg/kg/day) in combination with cyclophoshamide should be first choice of treatment in rapidly progressive OCP or anti-LN-332 MMP patients with impending blindness or airway obstruction due to pharyngeal and laryngeal involvement. Rituximab (anti-CD20 monoclonal antibody) and high-dose intravenous immunoglobulin are both used with good results in refractory MMP patients.[81,82]

EBA, classical MB phenotype, is known to be therapeutic refractory. Treatment with oral CS (0.5-1.0 mg/kg/day) is used in first instance and steroid sparing adjuvant is introduced simultaneously. The use of colchicine is described to be successfull, although the adverse effects of gastro-intestinal complaints can make it difficult for patients to achieve the prescribed dosage.[32] In our clinic we use azathioprine (2-3mg/kg/day), dapsone (100-200 mg/day), mycophenolate mofetil (2g/day) or mycophenolic acid (720 mg twice/day). Rituximab (anti-CD20 monoclonal antibody) and high-dose intravenous immunoglobulin are described to reduce symptoms in refractory EBA patients.[83-85] Inf EBA can be treated similar to BP. Dapsone is first choice of treatment in IgA EBA patients.

Aim of the thesis

The Centre for Blistering Diseases, Department of Dermatology, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands, is the national referral centre were patients with pemphigus and pemphigoid from the Netherlands and foreign countries are seen. The combination of high quality laboratory diagnostics and high standard clinical practice provides this centre to be leading in the field of autoimmune blistering diseases. The aim of this thesis is to provide more insight for the clinician in clinical symptoms, diagnosis and treatment in pemphigoid diseases, especially bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), anti-laminin-332 MMP (anti-LN-332 MMP) and epidermolysis bullosa acquisita (EBA).
To spread our knowledge of the serration pattern analysis and to encourage dermatologists and pathologists to use this assay in daily practice we developed in **chapter 2** an image-based online test and instruction video to test the learnability of n- and u-serrated DIF patterns before and after instruction. The purpose of this test is to demonstrate the importance of the serration pattern analysis as a diagnostic criterion in pemphigoid diseases and to show that adequate interpretation is learnable. For that reason our online nversusu-test is worldwide online available and free of charge.
In **chapter 3** we investigated the possible use of a new C7 ELISA for initial diagnosis of EBA. Furthermore we tested if C7 ELISA is a possible tool for monotoring disease activity, since this would provide the dermatologist with an assay to investigate the effect of medication on antibody levels.
Anti-LN-332 MMP is a rare disease and in **chapter 4** we focus on the immunopathological findings, clinical features and describe the therapeutic management in 10 patients with anti-LN-332 MMP to enhance the knowledge of this subtype of MMP and to describe the clinical differences compared to anti-p200 pemphigoid, which shows similarity in DIF serration pattern analysis (n-serrated pattern) and SSS (dermal binding).
In **chapter 5** we describe 15 patients selected over the period 2002-2012 from the biobank of our Centre for Blistering Diseases with immunopathological findings of BP, intense pruritus, and no development of blisters and define clinical features and treatment of this group of patients.
In **chapter 6** we investigate the efficacy and adverse effects of whole body topical clobetasol propionate cream application in patients with mild or severe BP and we also focus on the systemic effects of this treatment.

References

1 Bertram F, Brocker EB, Zillikens D et al. Prospective analysis of the incidence of autoimmune bullous disorders in Lower Franconia, Germany. J Dtsch Dermatol Ges 2009; 7: 434-40.

2 Gudi VS, White MI, Cruickshank N et al. Annual incidence and mortality of bullous pemphigoid in the Grampian Region of North-east Scotland. Br J Dermatol 2005; 153: 424-7.

3 Joly P, Baricault S, Sparsa A et al. Incidence and mortality of bullous pemphigoid in France. J Invest Dermatol 2012; 132: 1998-2004.

4 Langan SM, Smeeth L, Hubbard R et al. Bullous pemphigoid and pemphigus vulgaris-incidence and mortality in the UK: population based cohort study. Bmj 2008; 337: a180.

5 Schmidt E, Zillikens D. Pemphigoid diseases. Lancet 2013: 320–32.

6 Cortes B, Marazza G, Naldi L et al. Mortality of bullous pemphigoid in Switzerland: a prospective study. Br J Dermatol 2011; 165: 368-74.

7 Bastuji-Garin S, Joly P, Lemordant P et al. Risk factors for bullous pemphigoid in the elderly: a prospective case-control study. J Invest Dermatol 2011; 131: 637-43.

8 Langan SM, Groves RW, West J. The relationship between neurological disease and bullous pemphigoid: a population-based case-control study. J Invest Dermatol 2011; 131: 631-6.

9 Kasperkiewicz M, Zillikens D, Schmidt E. Pemphigoid diseases: pathogenesis, diagnosis, and treatment. Autoimmunity 2013; 45: 55-70.

10 Murrell DF, Daniel BS, Joly P et al. Definitions and outcome measures for bullous pemphigoid: recommendations by an international panel of experts. J Am Acad Dermatol 2011; 66: 479-85.

11 Di Zenzo G, Della Torre R, Zambruno G et al. Bullous pemphigoid: from the clinic to the bench. Clin Dermatol 2012; 30: 3-16.

12 Alonso-Llamazares J, Rogers RS, 3rd, Oursler JR et al. Bullous pemphigoid presenting as generalized pruritus: observations in six patients. Int J Dermatol 1998; 37: 508-14.

13 Feliciani C, Caldarola G, Kneisel A et al. IgG autoantibody reactivity against bullous pemphigoid (BP) 180 and BP230 in elderly patients with pruritic dermatoses. Br J Dermatol 2009; 161: 306-12.

14 Lamb PM, Abell E, Tharp M et al. Prodromal bullous pemphigoid. Int J Dermatol 2006; 45: 209-14.

15 Massa MC, Connolly SM. Bullous pemphigoid with features of prurigo nodularis. Arch Dermatol 1982; 118: 937-9.

16 Tashiro H, Arai H, Hashimoto T et al. Pemphigoid nodularis: two case studies and analysis of autoantibodies before and after the development of generalized blistering. J Nippon Med Sch 2005; 72: 60-5.

17 Wolf R, Ophir J, Dechner E. Nonbullous bullous pemphigoid. Int J Dermatol 1992; 31: 498-500.

18 Safa G, Darrieux L. Nonbullous pemphigoid treated with doxycycline monotherapy: report of 4 cases. J Am Acad Dermatol 2011; 64: e116-8.

19 Strohal R, Rappersberger K, Pehamberger H et al. Nonbullous pemphigoid: prodrome of bullous pemphigoid or a distinct pemphigoid variant? J Am Acad Dermatol 1993; 29: 293-9.
20 Chan LS, Ahmed AR, Anhalt GJ et al. The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenic factors, medical treatment, and prognostic indicators. Arch Dermatol 2002; 138: 370-9.
21 Thorne JE, Anhalt GJ, Jabs DA. Mucous membrane pemphigoid and pseudopemphigoid. Ophthalmology 2004; 111: 45-52.
22 Tauber J, Jabbur N, Foster CS. Improved detection of disease progression in ocular cicatricial pemphigoid. Cornea 1992; 11: 446-51.
23 Egan CA, Lazarova Z, Darling TN et al. Anti-epiligrin cicatricial pemphigoid: clinical findings, immunopathogenesis, and significant associations. Medicine (Baltimore) 2003; 82: 177-86.
24 Wozniak K, Waszczykowska E, Hashimoto T et al. Anti-epiligrin cicatricial pemphigoid initially limited to the upper respiratory tract. Br J Dermatol 2006; 154: 779-81.
25 Shipman AR, Ali I, Murrell DF et al. Mucous membrane pemphigoid: are laminin 5 antibodies a risk factor for laryngeal involvement? J Eur Acad Dermatol Venereol 2009; 23: 169-70.
26 Egan CA, Yancey KB. The clinical and immunopathological manifestations of anti-epiligrin cicatricial pemphigoid, a recently defined subepithelial autoimmune blistering disease. Eur J Dermatol 2000; 10: 585-9.
27 Egan CA, Lazarova Z, Darling TN et al. Anti-epiligrin cicatricial pemphigoid and relative risk for cancer. Lancet 2001; 357: 1850-1.
28 Sadler E, Lazarova Z, Sarasombath P et al. A widening perspective regarding the relationship between anti-epiligrin cicatricial pemphigoid and cancer. J Dermatol Sci 2007; 47: 1-7.
29 Matsushima S, Horiguchi Y, Honda T et al. A case of anti-epiligrin cicatricial pemphigoid associated with lung carcinoma and severe laryngeal stenosis: review of Japanese cases and evaluation of risk for internal malignancy. J Dermatol 2004; 31: 10-5.
30 Dainichi T, Koga H, Tsuji T et al. From anti-p200 pemphigoid to anti-laminin gamma1 pemphigoid. J Dermatol 2010; 37: 231-8.
31 Vafia K, Groth S, Beckmann T et al. Pathogenicity of autoantibodies in anti-p200 pemphigoid. PLoS One 2012; 7: e41769.
32 Ishii N, Hamada T, Dainichi T et al. Epidermolysis bullosa acquisita: what's new? J Dermatol 2010; 37: 220-30.
33 Buijsrogge JJ, Diercks GF, Pas HH et al. The many faces of epidermolysis bullosa acquisita after serration pattern analysis by direct immunofluorescence microscopy. Br J Dermatol 2012; 165: 92-8.

34 Borradori L, Sonnenberg A. Hemidesmosomes: roles in adhesion, signaling and human diseases. Curr Opin Cell Biol 1996; 8: 647-56.
35 Borradori L, Sonnenberg A. Structure and function of hemidesmosomes: more than simple adhesion complexes. J Invest Dermatol 1999; 112: 411-8.
36 Jones JC, Hopkinson SB, Goldfinger LE. Structure and assembly of hemidesmosomes. Bioessays 1998; 20: 488-94.
37 Stanley JR, Hawley-Nelson P, Yuspa SH et al. Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. Cell 1981; 24: 897-903.
38 Giudice GJ, Emery DJ, Diaz LA. Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP180. J Invest Dermatol 1992; 99: 243-50.
39 Labib RS, Anhalt GJ, Patel HP et al. Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. J Immunol 1986; 136: 1231-5.
40 Giudice GJ, Emery DJ, Zelickson BD et al. Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. J Immunol 1993; 151: 5742-50.
41 Skaria M, Jaunin F, Hunziker T et al. IgG autoantibodies from bullous pemphigoid patients recognize multiple antigenic reactive sites located predominantly within the B and C subdomains of the COOH-terminus of BP230. J Invest Dermatol 2000; 114: 998-1004.
42 Domloge-Hultsch N, Gammon WR, Briggaman RA et al. Epiligrin, the major human keratinocyte integrin ligand, is a target in both an acquired autoimmune and an inherited subepidermal blistering skin disease. J Clin Invest 1992; 90: 1628-33.
43 Aumailley M, Bruckner-Tuderman L, Carter WG et al. A simplified laminin nomenclature. Matrix Biol 2005; 24: 326-32.
44 Niessen CM, Hogervorst F, Jaspars LH et al. The alpha 6 beta 4 integrin is a receptor for both laminin and kalinin. Exp Cell Res 1994; 211: 360-7.
45 Rousselle P, Keene DR, Ruggiero F et al. Laminin 5 binds the NC-1 domain of type VII collagen. J Cell Biol 1997; 138: 719-28.
46 Sakai LY, Keene DR, Morris NP et al. Type VII collagen is a major structural component of anchoring fibrils. J Cell Biol 1986; 103: 1577-86.
47 Woodley DT, Briggaman RA, O'Keefe EJ et al. Identification of the skin basement-membrane autoantigen in epidermolysis bullosa acquisita. N Engl J Med 1984; 310: 1007-13.
48 Keene DR, Sakai LY, Lunstrum GP et al. Type VII collagen forms an extended network of anchoring fibrils. J Cell Biol 1987; 104: 611-21.

49 Chen M, Keene DR, Costa FK et al. The carboxyl terminus of type VII collagen mediates antiparallel dimer formation and constitutes a new antigenic epitope for epidermolysis Bullosa acquisita autoantibodies. J Biol Chem 2001; 276: 21649-55.
50 Mayuzumi M, Akiyama M, Nishie W et al. Childhood epidermolysis bullosa acquisita with autoantibodies against the noncollagenous 1 and 2 domains of type VII collagen: case report and review of the literature. Br J Dermatol 2006; 155: 1048-52.
51 Ishii N, Yoshida M, Hisamatsu Y et al. Epidermolysis bullosa acquisita sera react with distinct epitopes on the NC1 and NC2 domains of type VII collagen: study using immunoblotting of domain-specific recombinant proteins and postembedding immunoelectron microscopy. Br J Dermatol 2004; 150: 843-51.
52 Ishii N, Yoshida M, Ishida-Yamamoto A et al. Some epidermolysis bullosa acquisita sera react with epitopes within the triple-helical collagenous domain as indicated by immunoelectron microscopy. Br J Dermatol 2009; 160: 1090-3.
53 Vodegel RM, Jonkman MF, Pas HH et al. U-serrated immunodeposition pattern differentiates type VII collagen targeting bullous diseases from other subepidermal bullous autoimmune diseases. Br J Dermatol 2004; 151: 112-8.
54 Borradori L BP, Pemphigoid group. bullous pemphigoid, cicatricial pemphigoid. epidermolysis bullosa acquisita, Bolognia JL, Jorizzo JL, Rapini London: Dermatology, Elsevier Science edn., 2006.
55 Gupta R, Woodley DT, Chen M. Epidermolysis bullosa acquisita. Clin Dermatol 2013; 30: 60-9.
56 Lazarova Z, Sitaru C, Zillikens D et al. Comparative analysis of methods for detection of anti-laminin 5 autoantibodies in patients with anti-epiligrin cicatricial pemphigoid. J Am Acad Dermatol 2004; 51: 886-92.
57 Yancey KB, Kirtschig G, Yee C et al. Studies of patients with anti-epiligrin cicatricial pemphigoid. J Dermatol 1995; 22: 829-35.
58 Jordon RE, Beutner EH, Witebsky E et al. Basement zone antibodies in bullous pemphigoid. Jama 1967; 200: 751-6.
59 Schmidt E, Skrobek C, Kromminga A et al. Cicatricial pemphigoid: IgA and IgG autoantibodies target epitopes on both intra- and extracellular domains of bullous pemphigoid antigen 180. Br J Dermatol 2001; 145: 778-83.
60 Setterfield J, Shirlaw PJ, Kerr-Muir M et al. Mucous membrane pemphigoid: a dual circulating antibody response with IgG and IgA signifies a more severe and persistent disease. Br J Dermatol 1998; 138: 602-10.
61 Gammon WR, Briggaman RA, Inman AO, 3rd et al. Differentiating anti-lamina lucida and anti-sublamina densa anti-BMZ antibodies by indirect immunofluorescence on 1.0 M sodium chloride-separated skin. J Invest Dermatol 1984; 82: 139-44.

62 Gammon WR, Kowalewski C, Chorzelski TP et al. Direct immunofluorescence studies of sodium chloride-separated skin in the differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita. J Am Acad Dermatol 1990; 22: 664-70.
63 Willsteed E, Bhogal BS, Black MM et al. Use of 1M NaCl split skin in the indirect immunofluorescence of the linear IgA bullous dermatoses. J Cutan Pathol 1990; 17: 144-8.
64 Domloge-Hultsch N, Bisalbutra P, Gammon WR et al. Direct immunofluorescence microscopy of 1 mol/L sodium chloride-treated patient skin. J Am Acad Dermatol 1991; 24: 946-51.
65 Pas HH. Immunoblot assay in differential diagnosis of autoimmune blistering skin diseases. Clin Dermatol 2001; 19: 622-30.
66 Schmidt E, Obe K, Brocker EB et al. Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. Arch Dermatol 2000; 136: 174-8.
67 Kim JH, Kim YH, Kim S et al. Serum levels of anti-type VII collagen antibodies detected by enzyme-linked immunosorbent assay in patients with epidermolysis bullosa acquisita are correlated with the severity of skin lesions. J Eur Acad Dermatol Venereol 2012; 27: e224-30.
68 Saleh MA, Ishii K, Kim YJ et al. Development of NC1 and NC2 domains of type VII collagen ELISA for the diagnosis and analysis of the time course of epidermolysis bullosa acquisita patients. J Dermatol Sci 2011; 62: 169-75.
69 Kim JH, Kim YH, Kim S et al. Serum levels of anti-type VII collagen antibodies detected by enzyme-linked immunosorbent assay in patients with epidermolysis bullosa acquisita are correlated with the severity of skin lesions. J Eur Acad Dermatol Venereol 2013; 27 (2): e224-30.
70 Vodegel RM, Kiss M, Cjm De Jong M et al. The use of skin substrates deficient in basement membrane molecules for the diagnosis of subepidermal autoimmune bullous disease. Eur J Dermatol 1998; 8: 83-5.
71 De Jong MC, Bruins S, Heeres K et al. Bullous pemphigoid and epidermolysis bullosa acquisita. Differentiation by fluorescence overlay antigen mapping. Arch Dermatol 1996; 132: 151-7.
72 Wojnarowska F, Kirtschig G, Highet AS et al. Guidelines for the management of bullous pemphigoid. Br J Dermatol 2002; 147: 214-21.
73 Joly P, Roujeau JC, Benichou J et al. A comparison of two regimens of topical corticosteroids in the treatment of patients with bullous pemphigoid: a multicenter randomized study. J Invest Dermatol 2009; 129: 1681-7.
74 Joly P, Roujeau JC, Benichou J et al. A comparison of oral and topical corticosteroids in patients with bullous pemphigoid. N Engl J Med 2002; 346: 321-7.
75 van Velsen SG, De Roos MP, Haeck IM et al. The potency of clobetasol propionate: serum levels of clobetasol propionate and adrenal function during therapy with 0.05% clobetasol propionate in patients with severe atopic dermatitis. J Dermatolog Treat; 23: 16-20.

76 Venning VA, Taghipour K, Mohd Mustapa MF et al. British Association of Dermatologists' guidelines for the management of bullous pemphigoid 2012. Br J Dermatol 2012; 167: 1200-14.
77 Gaitanis G, Alexis I, Pelidou SH et al. High-dose intravenous immunoglobulin in the treatment of adult patients with bullous pemphigoid. Eur J Dermatol; 22: 363-9.
78 Tripodi G, Risso M, Tenerini L et al. Drug-resistant bullous pemphigoid and inflammatory bowel disease in a pediatric case successfully treated by plasma exchange and extracorporeal photochemotherapy. J Clin Apher 2007; 22: 26-30.
79 Mazzi G, Raineri A, Zanolli FA et al. Plasmapheresis therapy in pemphigus vulgaris and bullous pemphigoid. Transfus Apher Sci 2003; 28: 13-8.
80 Kasperkiewicz M, Shimanovich I, Ludwig RJ et al. Rituximab for treatment-refractory pemphigus and pemphigoid: a case series of 17 patients. J Am Acad Dermatol; 65: 552-8.
81 Letko E, Miserocchi E, Daoud YJ et al. A nonrandomized comparison of the clinical outcome of ocular involvement in patients with mucous membrane (cicatricial) pemphigoid between conventional immunosuppressive and intravenous immunoglobulin therapies. Clin Immunol 2004; 111: 303-10.
82 Le Roux-Villet C, Prost-Squarcioni C, Alexandre M et al. Rituximab for patients with refractory mucous membrane pemphigoid. Arch Dermatol; 147: 843-9.
83 Segura S, Iranzo P, Martinez-de Pablo I et al. High-dose intravenous immunoglobulins for the treatment of autoimmune mucocutaneous blistering diseases: evaluation of its use in 19 cases. J Am Acad Dermatol 2007; 56: 960-7.
84 Schmidt E, Benoit S, Brocker EB et al. Successful adjuvant treatment of recalcitrant epidermolysis bullosa acquisita with anti-CD20 antibody rituximab. Arch Dermatol 2006; 142: 147-50.
85 Sadler E, Schafleitner B, Lanschuetzer C et al. Treatment-resistant classical epidermolysis bullosa acquisita responding to rituximab. Br J Dermatol 2007; 157: 417-9.

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The n-versus u-serration is a learnable criterion to differentiate pemphigoid from epidermolysis bullosa acquisita in direct immunofluorescence serration pattern analysis

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Abstract

Background Serration pattern analysis of direct immunofluorescence (DIF) allows differentiating epidermolysis bullosa acquisita (EBA) from other subtypes of pemphigoid. In daily practice its use is limited due to lack of experience and unfamiliarity.

Objectives To test the learnability of DIF serrated pattern recognition under groups with various a priori levels of competence.

Methods An online nversusu-test (www.nversusu.umcg.nl) was created, which contained 26 DIF images of the epidermal basement membrane zone (BMZ), IgG stained, and photographed with a magnification of 40x and 63x. All images represented patients with a form of subepidermal autoimmune bullous disease. Thirteen DIF images were presented before and thirteen DIF images after an instruction video about n- and u-serrated patterns. There were three options to choose from: n-serrated, u-serrated or undetermined. The test was completed by three groups of professionals: i) dermatology residents in training at the University Medical Centre Groningen, ii) International experts on bullous diseases, iii) dermatologists and pathologists who participated in the Groningen Blistering Course in the last 10 years.

Results Overall the number of correct answers of serration patterns was significantly higher after instruction than before instruction (median 9.0 correct answers vs. 11.0 correct answers, P<.001). Participants showed a mean improvement after instruction of 15.4% in the UMCG group (66.7% vs. 82.1%), 16.2% in the International expert group (67.2% vs. 83.4%) and 12.1% in the Blistering Course group (60.7% vs. 72.8%). The u-serrated pattern was better recognized than the n-serrated.

Conclusion Serration pattern analysis by DIF can be learned irrespective of background of expertise.

Introduction

Acquired subepidermal autoimmune blistering diseases (sAIBD) comprises bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), anti-laminin-332 MMP (anti-LN-332 MMP), anti-p200 pemphigoid, anti-plectin pemphigoid, linear IgA bullous dermatosis (LAD), pemphigoid gestationis (PG), lichen planus pemphigoides (LPP), and epidermolysis bullosa acquisita (EBA). Each subtype is characterized by circulating autoantibodies targeting components of the epidermal basement membrane zone (BMZ).[1] The main target antigens in BP, MMP, LAD, PG and LPP are the 180-kD antigen (BP180, BPAG2, or type XVII collagen), and the 230-kD antigen (BP230, BPAG1). These are components of the hemidesmosomal plaque, adhesion structures

that anchor the basal cells to the underlying BMZ.[1] Anti-LN-332 MMP shows circulating autoantibodies targeting LN-332 which connects hemidesmosomes to anchoring fibrils by interlinking integrin alpha6betha4 to type VII collagen.[2] EBA shows autoreactivity to the 290-kDa antigen (type VII collagen), the major structural component of the anchoring fibrils located in the BMZ.[3] In 2004, Vodegel et al. described the serration pattern analysis by routine direct immunofluorescence (DIF) showing linear n-serration or linear u-serration immunodepositions along the BMZ.[4] The u-serration pattern confirms the diagnosis EBA, and represents immunoglobulin depositions in upstanding arms ("grass") of the sublamina densa zone between the rootlets of basal keratinocytes.[3] In all other sAIBDs the antigens are located in the lamina lucida or above, so the immunodeposits follow the rootlets of the basal keratinocytes showing the n-serration pattern.[2,4] However since its first publication, DIF serration pattern analysis has found limited use, although the criterion is mentioned in textbooks[5], and in the forthcoming European guideline on AIBD. The limited use might be caused by uncertainty and lack of training of the IF microscopists. Aim of this study is to test the learnability of n- and u-serrated DIF patterns before and after instruction. Moreover, for knowledge transfer, our image-based online test and instruction video is available online free of charge. To display the importance of DIF serration pattern analysis as a diagnostic criterion in subtyping sAIBD, we first present two cases in which it had an important role in early diagnosis.

Report of cases

A 42-year old female presented with desquamative gingivitis with oral ulcerations (Fig 1-AI), intra-nasal crustae, symblepharon (Fig 1-AII), and laryngeal involvement without skin symptoms. DIF of a skin biopsy showed linear IgG deposition along the BMZ in the n-serrated pattern. Indirect immunofluorescence (IIF) of serum on monkey esophagus (MO) showed circulating anti-BMZ IgG and IIF on salt-split skin (SSS) revealed IgG binding to the dermal side of the split. The combination of mucosal symptoms, n-serrated pattern by DIF, and dermal binding by SSS was suggestive for anti-LN- 332 MMP (Fig 1-AIII). Diagnosis was confirmed by a positive ELISA against native LN-332

A 31-year old male presented with urticarial plaques on the trunk (Fig 1-BI), mechanobullous blistering on hands and feet, and milia on the dorsal side of the hands (Fig 1-BII). DIF showed linear IgG deposition along the BMZ in the u-serrated pattern. IIF on MO showed circulating IgG against the BMZ, and IIF on SSS showed IgG binding to the dermal side of the split. The combination of clinical symptoms, u-serrated pattern by DIF (Fig 1-BIII) and dermal binding of

SSS was suggestive for classic mechanobullous EBA. This diagnosis was confirmed by immunoblot demonstrating IgG binding the 290 kDa-antigen and a positive type VII collagen ELISA.

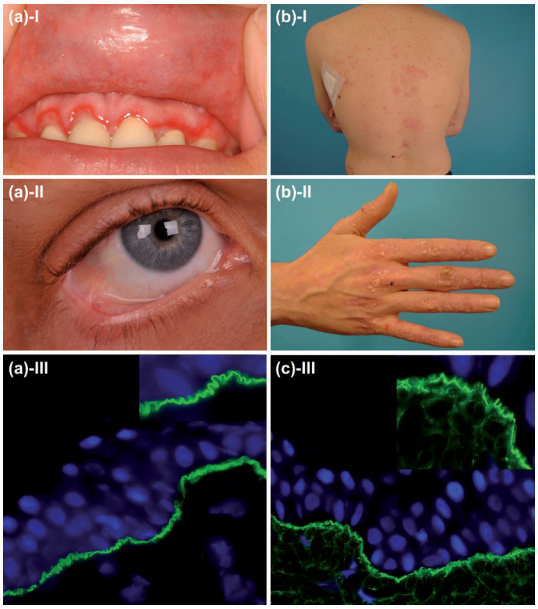

Figure 1: Clinical features and serration pattern analysis by direct immunofluorescence (DIF) in patient (A) anti-laminin-332 MMP and patient (B) epidermolysis bullosa acquisita, mechanobullous phenotype. (A-I) desquamative gingivitis, (A-II) conjunctivitis with symblepharon, (A-III) n-serrated pattern by DIF. (B-I) urticarial plaques on the back, (B-II) milia on the dorsal side of the hand, (B-III) u-serrated pattern by DIF.

Material and Methods

Study design

An image-based online test (www.nversusu.umcg.nl) was created, using LimeSurvey software.[6] The test included 26 DIF images of the BMZ, all biopsies were IgG stained and photographed with a 40x and 63x objective. First, the participant had to score thirteen DIF images, then an instruction video about n- and u-serrated pattern recognition was presented, and subsequently the participant had to score thirteen other DIF images. The short instruction video contained

instructions how to recognize the different serrated patterns with simple mnemonics: undulating n or u-serrated grass.[4] To reduce guessing and stay close to real practice the participant could also score undetermined, besides n-serrated and u-serrated.

Participants

Three groups of participants were selected who had a different a priori level of expertise on the subject: i) dermatology residents from the University Medical Centre Groningen (UMCG), ii) International experts in blistering diseases, who were selected by the network of MFJ derived from the EADV Task Force on Autoimmune Bullous Diseases and the Pre-International Investigative Dermatology Symposium on Autoimmune Bullous Diseases in Lübeck, 6-7 May 2013, iii) dermatologists and pathologists who had participated in the annual Dutch Blistering Course Groningen within the years 2005-2012.

Patients

Tissue samples of patients with sAIBD were collected from the biobank of the Groningen Centre for Blistering Diseases in the Netherlands from the period 2002-2012. The 26 patients included in this study were selected by previously confirmed positive DIF, showing a linear deposition pattern along the BMZ in a serrated pattern. The final diagnosis was based on clinical, routine laboratory, histological and immunopathological findings. Tissue samples with the n-serrated deposition pattern (n=13) included patients with one of the following diagnoses: bullous pemphigoid (BP; n=5), mucous membrane pemphigoid (MMP; n=5), or anti-laminin-332 mucous membrane pemphigoid (anti-LN-332 MMP; n=3). Tissue samples with the u-serrated deposition pattern included patients with epidermolysis bullosa acquisita (EBA; n=10). Three tissue samples showed an undetermined serration deposition pattern (BP; n=2 and EBA; n=1).

Direct immunofluorescensce microscopy (DIF)

The 4 mm IF punch biopsies were all derived from perilesional (erythematous) skin and had been transported prior to freezing in saline for 24 hours. Cryosections of 4 µm thickness were cut and mounted on polysine™ glass slides, air-dried for 30 minutes in front of a fan, and encircled with a hydrophobic emulsion pen (PAP pen, DAKO, Glostrup). The sections were then stained for 30 minutes with Fc-specific fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG.

The sections were examined with a Leica DMRA microscope (Leica, Wetzlar, Germany). Digital fluorescence images were acquired using a Leica DFC350FX camera (Leica, Wetzlar, Germany). Further image processing was done by Leica Application Suite software. The images of the serration pattern were validated until 100% concordance by two experts (GFHD and MFJ) and randomly assigned in the test before and after instruction. Participants had the possibility to leave their comments after completing the test.

Statistical analysis

Wilcoxon signed rank test was used to compare the scores of correct answers of serration patterns of the total group of participants before and after instruction. Scores of correct answers before and after instruction between the three groups of participants were compared using Kruskal-Wallis test. For all tests, two-sided p-values of less than 0.05 were considered to indicate statistical significance. All analyses were performed using commercially available software (SPSS, version 20, IBM).

Results

We sent 200 invitations to participate in the test; eighty-seven participants completed the online nversusu-test (Table 1). The UMCG group scored a response rate of 100% (33/33), the International experts scored 50% (19/38) response rate and in the Blistering Course participants 27% (35/128) performed the test.

The mean scores of correct answers before and after instruction of all participants were respectively 64.4% and 78.6% (P<.001). After instruction overall 60 participants (69%) improved in score, 10 participants (11.5%) worsened and 17 participants (19.5%) had the same score as before instruction. The mean improvement after instruction was 15.4% in the UMCG group (66.7% vs. 82.1%), 16.2% in the International expert group (67.2% vs. 83.4%) and 12.1% in the Blistering Course group (60.7% vs. 72.8%) (Fig 2). No significant difference in learnability was observed between groups.

DIF images with u-serrated patterns were recognized best, with a mean of 75% correct answers, whereas the n-serrated pattern was recognized with a mean of 69%. Remarkably, the undetermined patterns were well scored with correct answers in 68%. The best and least recognized DIF images of n-serrated, u-serrated and undetermined pattern are shown in figure 3. Participants scored 26% "undetermined" before the instruction video, compared to 18% "undetermined" after instruction.

		Before instruction (n=13 DIF images)		After instruction (n=13 DIF images)		P-value*
	No. of participants	No. of correct answers1	%	No. of correct answers1	%	
UMCG	33	8.7 ± 2.3	66.7	10.7 ± 1.3	82.1	<.001
Blistering Course	35	7.9 ± 2.2	60.7	9.5 ± 2.2	72.8	<.001
International Expert	19	8.7 ± 2.5	67.2	10.8 ± 1.5	83.4	.003
All	87	8.4 ± 2.3	64.4	10.2 ± 1.8	78.6	<.001

Table 1: Correct answers of serration pattern analysis by direct immunofluorescence of the test before and after instruction. Data are means ± SD, * P-value by Wilcoxon signed rank test

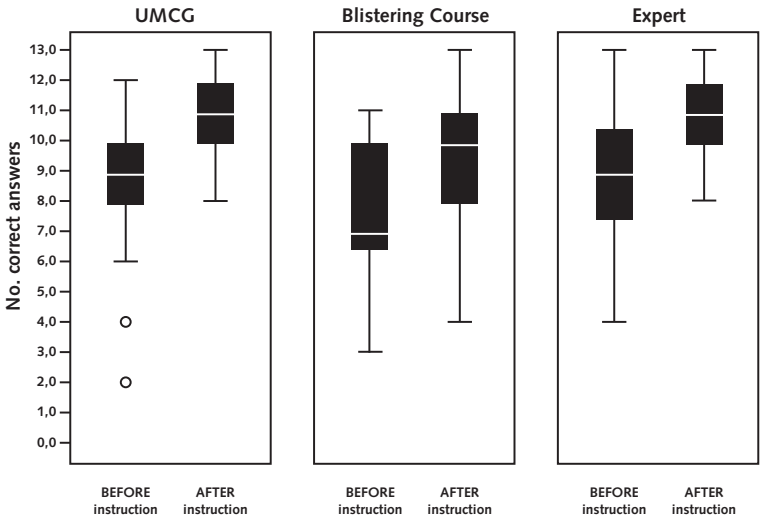

Figure 2: Total scores of the number correct answers of serration pattern analysis by direct immunofluorescence before and after instructions in three different groups.

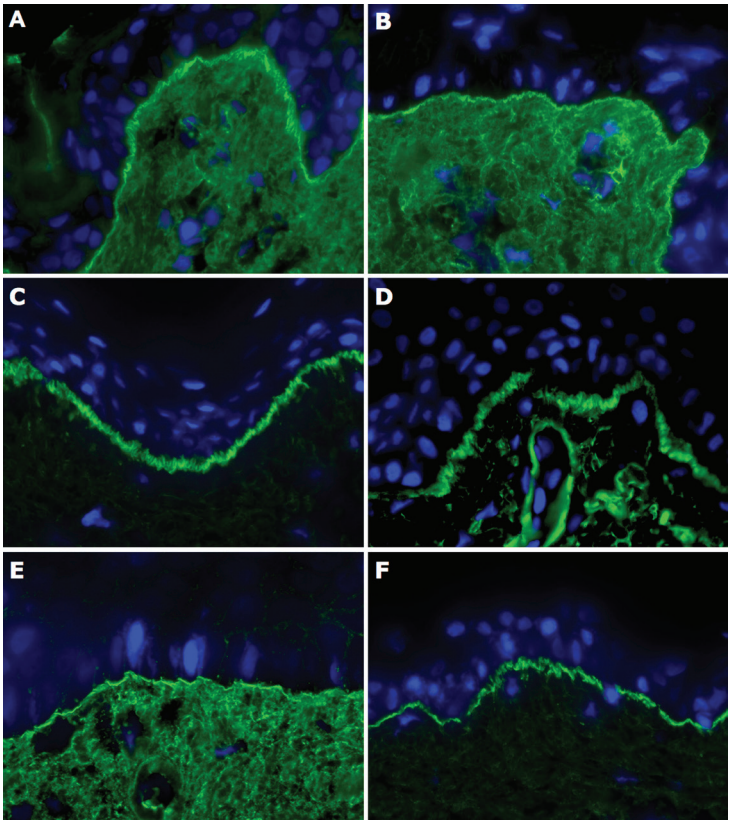

Figure 3: Best and least recognized serration pattern by direct immunofluorescence images: n-serrated (A: 88%; B: 47%), u-serrated (C: 96%; D: 40%) and undetermined (E: 88%; F: 44%). IgG stained, 63x magnification.

Comments of participants

Many participants commented positively on the high quality of the DIF images and serration patterns, a very clear instruction video and emphasized this is an easy way of learning about DIF serration patterns and encouraged us to make the test available to the public for teaching purposes.

Discussion

In this study we show that recognition of n- and u serration pattern by DIF is learnable irrespective of the level of expertise. The overall a priori level of serration pattern recognition was high with 69.2%. Significant improvement to 84.6% was reached by the online instruction video. No significant difference in a priori knowledge was observed between the three groups, what suggests that the International expert group, despite of their experience in sAIBD, is not more experienced in serration pattern analysis than the other participants in this study. The number of undetermined scores declined after viewing the instruction video, which suggests that participants are more confident in serration pattern analysis after instruction.

In serological negative cases or in cases in which IIF in combination with immunoblot and/or ELISA is not conclusive, serration pattern analysis of direct immunofluorescence (DIF) might disclose the diagnosis (Fig 4). Since more than half of the EBA patients do not have detectable circulating antibodies by either ELISA or SSS, DIF serration pattern analysis on perilesional skin biopsy is mandatory for diagnosis of an AIBD.[3,7] Buijsrogge et al. described a frequency of 5.5% EBA among patients with sAIBD when DIF serration pattern analysis is used.[3] We expect that many of those with inflammatory EBA are misdiagnosed as having pemphigoid when DIF serration pattern analysis is lacking.

For daily practice, adequate recognition of DIF serration pattern must meet the following standards: i) perilesional biopsies of non-scarring skin not exposed to topical corticosteroids, ii) transporting biopsies without freezing in saline for 24 hours, iii) cryosections of high quality (4 µm thickness or less), iv) lens objective of at least 40x.[4,8]

Limitations of our study are in the first place the possibility of selection bias, since we had a response rate of only 42%. Invited participants who were interested in serration pattern analysis might respond more often than invited participants with less experience in serration pattern analysis. Secondly, although the DIF images were of high quality standard, self assessment of the slides under a microscope is superior and might even give better results. Finally, in general it is important to realize that the serration pattern not always can be recognized, especially in mucosal biopsies.[4]

In conclusion, we show that serration pattern analysis by DIF is learnable. Our image-based online test and instruction video is available online free of charge (www.nversusu.umcg.nl).

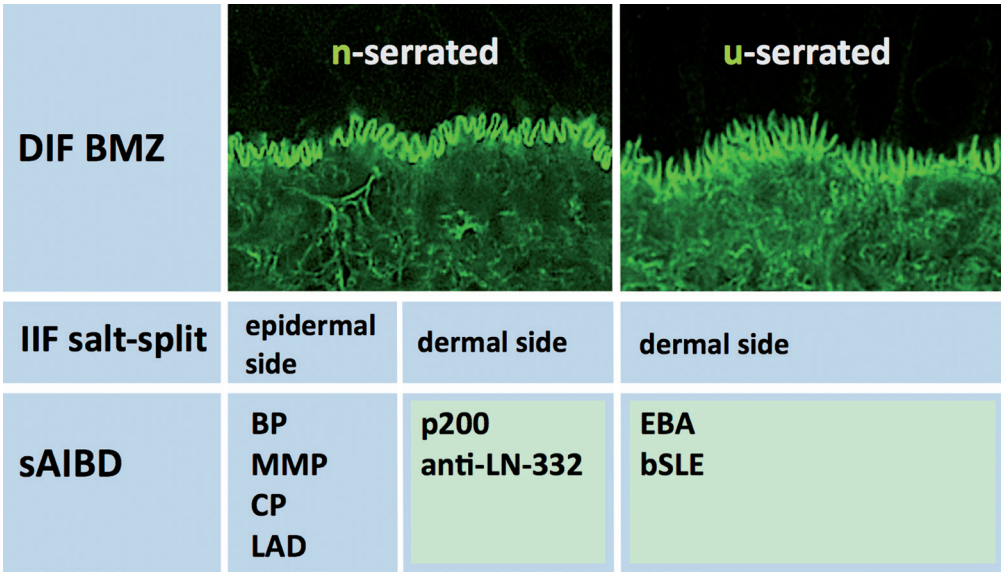

Figure 4: Overview of the serration pattern, IIF on salt split skin (SSS) and sAIBD; n-serrated in BP: bullous pemphigoid, MMP: mucous membrane pemphigoid, CP: cicatricial pemphigoid, LAD: linear IgA dermatosis, p200: anti-p200 pemphigoid and anti-LN-332: anti-laminin-332 mucous membrane pemphigoid. U-serrated in EBA: epidermolysis bullosa acquisita and bSLE: bullous systemic lupus erythematodus.

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REFERENCES

1 Schmidt E, Zillikens D. Pemphigoid diseases. Lancet 2013; 381: 320-32.

2 Terra JB, Pas HH, Hertl M et al. Immunofluorescence serration pattern analysis as a diagnostic criterion in antilaminin-332 mucous membrane pemphigoid: immunopathological findings and clinical experience in 10 Dutch patients. Br J Dermatol 2011; 165: 815-22.

3 Buijsrogge JJ, Diercks GF, Pas HH et al. The many faces of epidermolysis bullosa acquisita after serration pattern analysis by direct immunofluorescence microscopy. Br J Dermatol 2011; 165: 92-8.

4 Vodegel RM, Jonkman MF, Pas HH et al. U-serrated immunodeposition pattern differentiates type VII collagen targeting bullous diseases from other subepidermal bullous autoimmune diseases. Br J Dermatol 2004; 151: 112-8.

5 Borradori L BP, Pemphigoid group. bullous pemphigoid, cicatricial pemphigoid. epidermolysis bullosa acquisita, Bolognia JL, Jorizzo JL, Rapini London: Dermatology, Elsevier Science edn. 2006.

6 LimeSurvey Project Team / Carsten Schmitz (2012). / LimeSurvey: An Open Source survey tool /LimeSurvey Project Hamburg, Germany. URL http://www.limesurvey.org. In. 2012.

7 Gammon WR, Kowalewski C, Chorzelski TP et al. Direct immunofluorescence studies of sodium chloride-separated skin in the differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita. J Am Acad Dermatol 1990; 22: 664-70.

8 Vodegel RM, de Jong MC, Meijer HJ et al. Enhanced diagnostic immunofluorescence using biopsies transported in saline. BMC Dermatol 2004; 4: 10.

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Low sensitivity of type VII collagen ELISA in epidermolysis bullosa acquisita: serration pattern analysis on skin biopsy is required for diagnosis

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Abstract

Background Type VII collagen (coll VII) ELISA has been reported to have high sensitivity (>93%) and specificity (>96%) for diagnosing epidermolysis bullosa acquisita (EBA) patients who are seropositive by indirect immunofluorescence on salt-split skin (SSS).

Objectives To investigate the added value of coll VII ELISA in the laboratory diagnosis of SSS-positive and SSS-negative EBA and to correlate the ELISA index with disease episode.

Methods Coll VII ELISA was performed on banked sera of 28 EBA patients: 15 SSS-positive and 13 SSS-negative. Sera from healthy blood donors (n=17) and other autoimmune blistering diseases (n=29) served as controls. In four patients ELISA index was measured longitudinally. Serration pattern analysis by DIF was prospectively performed since 2000 and comprised 19 patients.

Results The sensitivity in the SSS-positive group was 80% whereas it was 23% in the SSS-negative group. In the prospective EBA subset it was 45%. The sensitivity of u-serration pattern analysis on skin biopsy was 89%. Ten (53%) of these cases were seronegative by both ELISA and SSS, and would have been missed by serum analysis alone. Of the 46 control sera one serum tested positive (specificity 97.8%). The coll VII ELISA correlated with disease activity over time in individual patients.

Conclusions Coll VII ELISA has limited added value in SSS-negative EBA cases. The ELISA test is very valuable in differentiating EBA from anti-laminin-332 MMP and anti-p200 pemphigoid and in its ability to serological monitor EBA patients. U-serration pattern analysis on IF skin biopsy is the gold standard for the diagnosis of EBA.

Introduction

Epidermolysis bullosa acquisita (EBA) is a subepidermal autoimmune blistering disease characterized by autoantibodies targeting type VII collagen (coll VII) that is the major structural component of the anchoring fibrils of the epidermal basement membrane zone (BMZ). Clinically EBA can present as either the classic mechanobullous phenotype or as the inflammatory phenotype that mimicks other pemphigoid diseases as bullous pemphigoid, mucous membrane pemphigoid or linear IgA dermatosis.[1] In the latter cases the diagnosis therefore relies on laboratory tests. In case of EBA immunofluorescence analysis of perilesional skin biopsies will show linear IgG BMZ deposits that have an unique u-serrated pattern in contrast to all other pemphigoid diseases where the deposits have a n-serrated pattern.[2,3] Analysis of serum by indirect immunofluorescence may show IgG binding to the floor of salt-split skin (SSS) substrate and immunoblotting

may reveal IgG binding the 290-kDa antigen.[4-7] In 1997 Chen et al. described a highly specific ELISA using the recombinant NC1 domain.[8] Recently new ELISA's for the detection of autoantibodies against coll VII have emerged. A commercial ELISA is available that has the recombinant NC1 and NC2 coll VII domains coated to the plate, and high sensitivity (>93%) and specificity (>96%) has been reported [9,10] A correlation between coll VII ELISA index and disease severity was also demonstrated.[10,11] Komorowski et al. used an ELISA plate containing only recombinant NC1 domain and reported a sensitivity of 98.7% and a specificity of 98.7%.[12] All these studies relied on sera that were positive by SSS analysis.
To investigate how the type VII coll ELISA would contribute to diagnose EBA in a normal routine setting, we tested the banked sera of our EBA patients including the sera that were negative by SSS. Time sequence correlations of disease episode and ELISA values were investigated in four patients.

Material and Methods

Patient sera

Sera from EBA patients (n=28) were retrospectively selected from our biobank (Table 1). For the sera collected after 2000 the diagnosis of EBA was based on the following criteria: subepidermal mucocutaneous blisters and the u-serrated pattern of linear IgG BMZ deposits (#9-19, #21-23, #26-28). If no biopsy was available or if the serration pattern could not be defined patients were included based on the presence of subepidermal mucocutaneous blisters and serum binding to the dermal side of SSS and recognizing the 290-kDa antigen on immunoblot (#20, 24 and 25). For the eight patients who were diagnosed with EBA before 2000, diagnosis was made for five by the combination of subepidermal mucocutaneous blisters, dermal SSS staining and positive immunoblot (#2-6). Furthermore two patients were included because of their classic generalized mechanobullous phenotype and dermal SSS staining (#7-8). One seronegative patient was included who had retrospectively been diagnosed with EBA in a case study (#1).[13] Of all EBA patients diagnosed before 2000, the serration patterns of the IgG deposition were retrospectively confirmed to be of the u-serrated type. As controls served sera from healthy blood donors (n=17) and from patients with other autoimmune blistering diseases (n=29).

Patient#	Sex	Age at diagnose	Pheno-type	DIF	MO	SSS	Immunoblot coll VII	Coll VII ELISA (index)
1	F	70	Inf-BP	u-serrated	-	-	-	+ (22)
2	M	46	Mb-cMB	u-serrated	-	dermal	+	-
3	F	74	Mb-BrPr	u-serrated	+	dermal	+	+ (73)
4	F	17	Inf-BP	u-serrated	+	dermal	+	+ (20)
5	F	64	Inf-BP	u-serrated	+	dermal	+	+ (45)
6	M	28	Inf-BP	u-serrated	+	dermal	+	+ (38)
7	M	67	Mb-cMB	u-serrated	+	dermal	-	-
8	M	26	Mb-cMB	u-serrated	+	dermal	-	+ (59)
9	F	53	Inf-BP	u-serrated	-	-	-	-
10	F	59	MB-cMB	u-serrated	-	dermal	+	+ (101)
11	F	31	MB-cMB	u-serrated	-	-	-	+ (10)
12	F	38	MB-cMB	u-serrated	+	dermal	-	-
13	M	84	Inf-Pru	u-serrated	-	-	-	-
14	M	31	MB-cMB	u-serrated	+	dermal	+	+ (101)
15	M	79	Inf-BP	u-serrated	-	-	-	-
16	F	34	MB-cMB	u-serrated	-	-	-	+ (52)
17	F	37	MB-cMB	u-serrated	+	dermal	-	+ (129)
18	F	15	MB-cMB	u-serrated	+	dermal	-	+ (94)
19	M	69	Inf-BP	u-serrated	-	-	-	-
20	F	87	Inf-MMP	undetermined	+	dermal	+	+ (58)
21	F	59	Inf-BP	u-serrated	-	-	-	-
22	F	62	Inf-BP	u-serrated	-	-	-	-
23	F	45	Mb-cMB	u-serrated	-	-	-	-
24	F	18	Inf-BP	na	-	dermal	+	+ (182)
25	F	33	MB-cMB	undetermined	-	dermal	+	+ (15)
26	F	81	MB-cMB	u-serrated	-	-	-	-
27	F	26	MB-cMB	u-serrated	-	-	-	-
28	F	29	MB-cMB	u-serrated	-	-	-	-

Table 1: Clinical and laboratory diagnostics of 28 patients with epidermolysis bullosa acquisita (#1-8 diagnosed retrospectively before 2000; # 9-28 diagnosed prospectively from 2000).MB, mechanobullous phenotype; cMB, classic mechanobullous; Inf, inflammatory phenotype; BP, bullous pemphigoid-like; MMP, mucous membrane pemphigoid-like; Pru, pruritus-like; M, male; F, female; DIF, serration pattern by direct immunofluorescence; MO, indirect immunofluorescence on monkey esophagus; SSS, indirect immunofluorescence on salt-split skin, +, positive; -, negative; n.a., not available

Type VII collagen ELISA

The anti-type VII collagen ELISA kit containing the recombinant NC1 and NC2 domains was used following the manufacturer's protocol (MBL, Nagoya, Japan).
Index values over six were considered positive.

Results

Fifteen of the 28 EBA sera (54%) had a positive ELISA index, median value 66.6 U/ml, range 10-182 U/ml. Of the 15 SSS-positive patients 12 (80%) had a positive ELISA index (Table 1).
To ascertain that the three with a negative coll VII ELISA (#2, #7, #12) did recognize type VII collagen we performed immunofluorescence knock-out analysis.[14] Two sera (#7, #12) did not bind to coll VII deficient skin, but did bind to laminin-332 deficient skin confirming the presence of anti-coll VII antibodies. Of the third serum (#2), which had the lowest titre (1+) by SSS, the binding could not be determined due to background. This serum however had a positive immunoblot for the 290-kDa antigen. Of the thirteen sera that tested negative by SSS, three (#1, #11, #16) tested positive in the ELISA (23%). Of the 46 controls one serum tested positive what gives a specificity of 97.8 %.
Since 2000 we prospectively performed serration pattern analysis. Of 19 patients we had biopsies available (#9-23, #25-28) and in 17 (89%) we identified the u-serrated pattern. For two cases the serration pattern was undeterminable, what for one case was due to the biopsy been taken from oral mucosa in which serration pattern analysis cannot be performed due to lack of overt basal cell rootlets.[3]
In this prospective EBA group (n=20; #9-28) coll VII ELISA was positive in nine cases (45%), and SSS was positive in eight cases (40%). Ten cases (50%) were seronegative by ELISA and SSS and would therefore have been missed if only serum tests had been used. The real sensitivity of ELISA and SSS for diagnosing EBA is therefore respectively 45% and 40% and combining those gives 50% sensitivity.
For 4 patients we could correlate coll VII ELISA index values and disease episode in relation to medication (Fig 1)

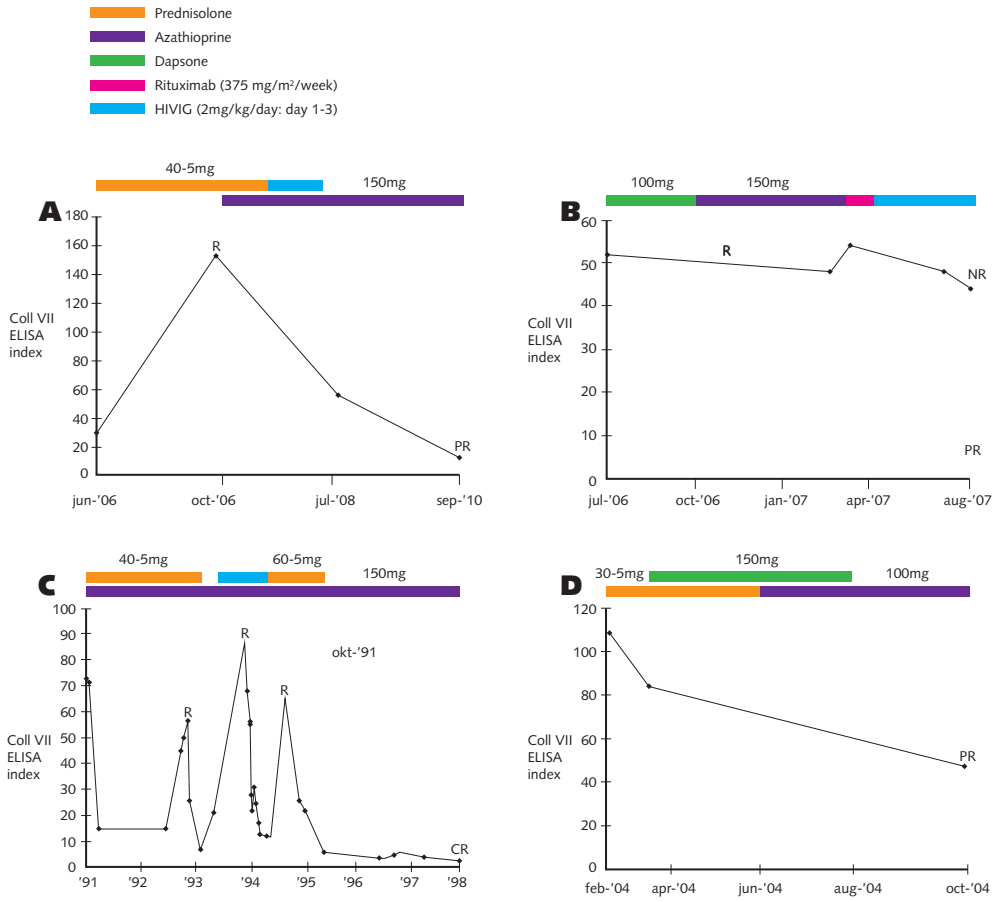

Figure 1: Type VII collagen ELISA indices in relation to medication and disease episode in four patients with epidermolysis bullosa acquisita (1A:#17; 1B:#16; 1C:#3; 1D:#14). The dose of each medication is listed above the line. R: relapse; PR: partial remission: CR: complete remission; NR: no response; HIVIG: human intravenous immunoglobulin; Coll VII: type VII collagen

Discussion

For our SSS-positive sera we found a sensitivity of 80% which is lower than the >93% found in earlier studies.[9,11,12] The three patients that tested negative clinically fitted the diagnosis mechanobullous EBA. Since the knock-out immunofluorescence analysis demonstrated that at least two of the three sera did contain anti-coll VII antibodies, the conclusion must be that the recognized epitope(s) are not available on the ELISA plate

Interestingly, in our SSS-negative group 3 of 13 patients sera tested positive in the ELISA, although two sera had low ELISA titres. Chen et al. also found that SSS-negative patients tested positive in their ELISA.[8]

In serological positive patients, SSS IgG dermal binding in combination with positive immunoblot or ELISA, is sufficient to diagnose EBA. Since more than half of the EBA patients do not have detectable circulating antibodies by either SSS, immunoblot or ELISA, DIF serration pattern analysis on perilesional skin biopsy is mandatory for diagnosis in such cases. According to our results 89% of the EBA patients can be diagnosed by this method. If the serration pattern is undefinable, ELISA will find in 45% of such cases anti-coll VII autoantibodies. For the remaining cases that are positive by SSS analysis but negative by coll VII ELISA, a diagnosis of EBA can be reached by immunoblot and IF knock-out analysis.

We had consecutive sera of four patients and confirmed earlier studies where a correlation of ELISA and disease activity was described.[9,10] Thus the coll VII ELISA test is a useful laboratory parameter for monitoring clinical activity. Another advantage of the ELISA test is that the diagnosis of EBA is direct and excludes anti-laminin-332 MMP or anti-p200 pemphigoid.

In conclusion, coll VII ELISA is a valuable confirmative laboratory test for diagnosing and monitoring EBA, but it does not replace a skin biopsy for serration pattern analysis when suspecting EBA.

Acknowledgements

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References

1 Buijsrogge JJ, Diercks GF, Pas HH et al. The many faces of epidermolysis bullosa acquisita after serration pattern analysis by direct immunofluorescence microscopy. Br J Dermatol 2011; 165: 92-8.

2 Lehman JS, Camilleri MJ, Gibson LE. Epidermolysis bullosa acquisita: concise review and practical considerations. Int J Dermatol 2009; 48: 227-35; quiz 35-6.

3 Vodegel RM, Jonkman MF, Pas HH et al. U-serrated immunodeposition pattern differentiates type VII collagen targeting bullous diseases from other subepidermal bullous autoimmune diseases. Br J Dermatol 2004; 151: 112-8.

4 Gammon WR, Kowalewski C, Chorzelski TP et al. Direct immunofluorescence studies of sodium chloride-separated skin in the differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita. J Am Acad Dermatol 1990; 22: 664-70.

5 Ishii N, Yoshida M, Hisamatsu Y et al. Epidermolysis bullosa acquisita sera react with distinct epitopes on the NC1 and NC2 domains of type VII collagen: study using immunoblotting of domain-specific recombinant proteins and postembedding immunoelectron microscopy. Br J Dermatol 2004; 150: 843-51.

6 Woodley DT. Immunofluorescence on salt-split skin for the diagnosis of epidermolysis bullosa acquisita. Arch Dermatol 1990; 126: 229-31.

7 Woodley DT, Briggaman RA, O'Keefe EJ et al. Identification of the skin basement-membrane autoantigen in epidermolysis bullosa acquisita. N Engl J Med 1984; 310: 1007-13.

8 Chen M, Chan LS, Cai X et al. Development of an ELISA for rapid detection of anti-type VII collagen autoantibodies in epidermolysis bullosa acquisita. J Invest Dermatol 1997; 108: 68-72.

9 Saleh MA, Ishii K, Kim YJ et al. Development of NC1 and NC2 domains of type VII collagen ELISA for the diagnosis and analysis of the time course of epidermolysis bullosa acquisita patients. J Dermatol Sci 2011; 62: 169-75.

10 Kim JH, Kim YH, Kim S et al. Serum levels of anti-type VII collagen antibodies detected by enzyme-linked immunosorbent assay in patients with epidermolysis bullosa acquisita are correlated with the severity of skin lesions. J Eur Acad Dermatol Venereol 2013; 27 (2); e224-30.

11 Marzano AV, Cozzani E, Fanoni D et al. Diagnosis And Disease Severity Assessment Of Epidermolysis Bullosa Acquisita By Elisa For Anti-Type Vii Collagen Autoantibodies: An Italian Multicenter Study. Br J Dermatol 2013; 168(1): 80-4.

12 Komorowski L, Muller R, Vorobyev A et al. Sensitive and specific assays for routine serological diagnosis of epidermolysis bullosa acquisita. J Am Acad Dermatol 2012; 15.

13 Buijsrogge JJ, de Jong MC, Meijer HJ et al. Inflammatory epidermolysis bullosa acquisita with coexistent IgA antibodies to plectin. Clin Exp Dermatol 2005; 30: 531-4.
14 Vodegel RM, Kiss M, Cjm De Jong M et al. The use of skin substrates deficient in basement membrane molecules for the diagnosis of subepidermal autoimmune bullous disease. Eur J Dermatol 1998; 8: 83-5.

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IF serration pattern analysis as a diagnostic criterion in anti-laminin-332 mucous membrane pemphigoid - immunopathological findings and clinical experience in 10 Dutch patients-

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Abstract

Background Anti-laminin-332 mucous membrane pemphigoid (anti-LN-332 MMP) is a chronic subepidermal blistering disease characterized by IgG anti-epidermal basement membrane zone (BMZ) autoantibodies against laminin-332 (LN-332). Patients with anti-LN-332 MMP have an increased relative risk of malignancy. For diagnosis of anti-LN-332 MMP difficult to obtain laboratory techniques are needed.

Objectives To incorporate direct immunofluorescence (DIF) serration pattern analysis of IgG depositions in the diagnostic criteria of anti-LN-332 MMP.

Methods Patients that met our revised inclusion criteria for anti-LN-332 MMP were selected from our biobank over the period 1997-2009. Inclusion criteria were clinical symptoms, DIF serration pattern analysis, indirect immunofluorescence on salt-split skin, and antigen-specificity analysis of the serum including immunoblotting and/or immunoprecipitation and/or ELISA against native LN-332.

Results Ten patients met the inclusion criteria. A malignancy was found in two patients (20%). In all patients in whom it was performed (n=9), DIF showed linear IgG deposition along the BMZ in an n-serrated pattern. Nine sera reacted by salt-split skin analysis and bound to the dermal side of the split skin. ELISA against native LN-332 was positive in 77.8% of the tested sera.

Conclusions Anti-LN-332 MMP can clinically resemble other forms of pemphigoid. Although state of the art laboratory diagnostics are necessary for definite diagnosis, the combination of simple DIF serration pattern and IIF salt-split skin analysis will exclude other forms of MMP and epidermolysis bullosa acquisita from the differential diagnosis. Because of the increased risk for malignancy patients should be thoroughly oncologically screened.

Introduction

Domloge-Hultsch et al. were the first to describe a novel autoimmune subepidermal blistering disease with autoantibodies that bind epiligrin: anti-epiligrin cicatricial pemphigoid. Epiligrin appeared to be a mixture of laminin 5, now named laminin-332 (LN-332), laminin-6 (LN-311), and laminin-7 (LN-321).[1,2] LN-332 is a heterotrimeric protein consisting of α3, β3 and γ2 subunits (laminin $\alpha3\beta3\gamma2$). LN-332 is present in the basal lamina of various epithelia including stratified squamous epithelium, and connects hemidesmosomes to anchoring fibrils by interlinking integrin $\alpha6\beta4$ to type VII collagen.[3,4] In most patients with anti-LN-332 mucous membrane pemphigoid (anti-LN-332 MMP) the IgG autoantibodies predominantly target the LN-332 α3 subunit, although IgG autoantibodies targeting the β3 or γ2 subunit have also been described.[5,6]

The α3A subunit splice variant of LN-332 is similar to that in LN-311, and LN-321, implying that these laminin trimers are also crosstargets.[7] Since LN-311, and LN-321 are relatively weakly expressed in the BMZ, and their β1 and β2 subunits have not been reported in autoimmune disease, for convenience of simplicity only LN-332 is assumed to be the autoimmune antigen in anti-LN-332 MMP.

Anti-LN-332 MMP is clinically indistinguishable from other forms of MMP and presents with involvement of the mucosal surfaces of the mouth, eyes, nasopharynx, oropharynx, larynx and anogenital region.[8-11] Approximately 5 to 20% of all MMP patients show circulating IgG autoantibodies against LN-332.[5,12] In most patients the skin is also involved, but usually less severe. An increased relative risk of cancer has been reported.[5,13-17]

The current diagnosis of anti-LN-332 MMP is based on criteria described previously: (i) chronic subepithelial blistering lesions of mucous membranes and skin, (ii) direct immunofluorescence (DIF) microscopy showing linear deposits of IgG with or without C3c along the BMZ, (iii) indirect immunofluorescence (IIF) microscopy showing IgG autoantibodies binding to the dermal side salt-split human skin, and (iv) circulating IgG anti-BMZ autoantibodies that immunoprecipate LN-332 from human keratinocyte extracts.[18-20]

In this study we present clinical and immunopathological findings in a cohort of 10 Dutch patients. Our data demonstrate a new algorithm to diagnose anti-LN-332 MMP based on the combination of clinical symptoms, state-of-the-art laboratory diagnostics and multidisciplinary cooperation. We demonstrate an important role for serration pattern analysis of the deposited IgG.

Material and Methods

Patients

Patients were selected from our database over the period 1997-2009. The following inclusion criteria for anti-LN-332 MMP were present: (i) subepidermal blisters and/or erosions on mucosal surfaces (ii) linear deposits of IgG along the BMZ in an n-serrated pattern – this separates anti-LN-332 MMP from epidermolysis bullosa aquisita (EBA) that is exclusively characterized by an u-serrated IgG deposition pattern –, (iii) circulating IgG autoantibodies that bind to the dermal side of 1-mol/L NaCL-split human skin – this separates anti-LN-332 MMP from other pemphigoid diseases of which the serum binds the roof of the split as for anti-BP180 MMP –, (iv) autoantibodies binding to LN-332 by either immunoblot, LN-332 ELISA, or immunoprecipitation; – this separates anti-LN-332 MMP from anti-p200 pemphigoid as both diseases

are indistinguishable by DIF and IIF: both have an n-serrated IgG deposition in skin and both have serum antibodies that bind the floor of salt-split skin .[21] The characteristics of the included patients are depicted in table 1.

Patient (#)	Sex	Age (years)	Affected mucous membranes	Skin	Therapy	Outcome
1	m	27	oral, ocular, nasal, pharyngeal, laryngeal, anogenital	localized	prednisolone, dexamethasone, cyclophosphamide	relapse, suicide
2	m	25	oral, nasal, pharyngeal, laryngeal	-	prednisolone, dexamethasone, doxycycline/nicotinamide azathioprine, cyclophosphamide	died after removal tracheacanule
3	f	64	oral, pharyngeal	generalized	prednisolone, dexamethasone, doxycycline/nicotiamide, dapsone	clinical remission. sporadic relapse: prednisolone (2010)
4	f	65	oral, ocular, nasal, pharyngeal	localized	prednisolone, dexamethasone, azathioprine, intravenous immunoglobuline	clinical remission with azathioprine 100 mg/day
5	f	42	oral, ocular, nasal, pharyngeal, laryngeal	-	prednisolone, dexamethasone, azathioprine, cyclophosphamide, mycophenolic acid, dapsone	clinical remission with 5 mg prednisolone
6	f	56	oral, anogenital	localized	prednisolone, azathioprine	clinical remission with azathioprine 100 mg/day
7	f	28	oral, ocular	generalized	prednisolone, azathioprine	clinical remission without medicin
8	m	56	ocular	localized	unknown	† lungcarcinoma
9	m	78	ocular, nasal	generalized	prednisolone	clinical remission without medicin
10	m	10	oral, ocular, nasal, pharyngeal	localized	prednisolone, dapsone, ciclosporin, cyclophosphamide	clinical remission after therapy lung carcinoma

Table 1: Clinical characteristics in 10 patients with anti-LN-332 MMP

Immunofluorescence microscopy

The technical procedure for immunofluorescence staining has been described in detail before.[21] Non-lesional skin, perilesional skin and/or mucosa biopsies were studied by DIF for BMZ deposits of IgG, IgA, IgM and C3c. The deposition pattern was typed as true linear, n-serrated or u-serrated. Serum samples were analysed by indirect immunofluorescence on both monkey esophagus and 1 mol/L NaCl-split human skin substrate.

Immunoblot and immunoprecipitation

Immunoblot analysis was performed with extract of cultured human keratinocytes as described before.[22] Immunoprecipitation was performed with extracts and medium from normal human keratinocytes radiolabeled with sulfur35–methionine.[23,24]

LN-332 ELISA

The LN-332 ELISA has been performed as described before.[25]

Results

Clinical manifestations

Ten patients were included. Eight of them had been treated by us, and of two patients' blood and tissue samples had been sent to us from other hospitals for laboratory diagnostics. Five patients were male and five patients were female; mean age at onset of symptoms was 50 years (range 23-78 years). The mean age at date of diagnosis was 51.1 years (range 25-78 years). Delay in diagnosis ranged from 2 to 48 months. In one exceptional case the patient had complaints of epistaxis and oral ulcerations for four years, before she was referred to our hospital and was diagnosed anti-LN-332 MMP. The mean follow up of the patients was 8.3 years (range 3-14 years).

The clinical symptoms included involvement of the skin (n=8), oral (n=8), nasal (n=6), ocular (n=7), pharyngeal (n=6), laryngeal (n=3) and anogenital (n=2) regions respectively (Fig 1 and table 1).

Skin symptoms consisted of recurrent bullae and erosions on the trunk and extremities. In cases #3, 7 and 9 blistering was generalized. Oral lesions consisted of desquamative gingivitis and ulcerations, sometimes leading to pain hampering oral intake.

Nasal involvement was apparent by epistaxis and intra-nasal crustae. Ocular involvement led to conjunctivitis, eye dryness with a burning sensation, and symblepharon. In patient #1, pannus formation resulted in blindness of one eye. In three patients the larynx was involved which led to hoarseness and dyspnoea because of ulcerations and erosions on the vocal folds (Fig 2). In patient #2 cicatrizing laryngeal lesions resulted in airway stenosis - he needed a life saving tracheotomy.

A malignancy was found in two out of ten patients (20%): both had adenocarcinoma of the lung. In one patient the lung carcinoma was diagnosed one year after the start of the mucocutaneous blistering. Interestingly, complete clinical remission of the anti-LN-332 MMP was seen after complete remission of the lung carcinoma. Detailed information on the other patient was not available.

Eight of ten patients were treated at our clinic. All eight patients received systemic corticosteroids (prednisolone 40-60 mg/day). In four patients dexamethasone pulse therapy (three consecutive days 300 mg a day orally or 200 mg a day intravenously) was added in one or two cycles because of unresponsiveness to prednisolone (#1-4) or severe laryngeal stridor (#5). In one patient clinical remission was achieved after dexamethasone pulse therapy. In the other four patients clinical symptoms improved dramatically, although adjuvant immunosuppressive drugs was needed after months. Other adjuvant immunosuppressive drugs used were dapsone, azathioprine, mycophenolic acid/mycophenolate mofetil, cyclophosphamide, and intravenous immunoglobulins (Table 1).

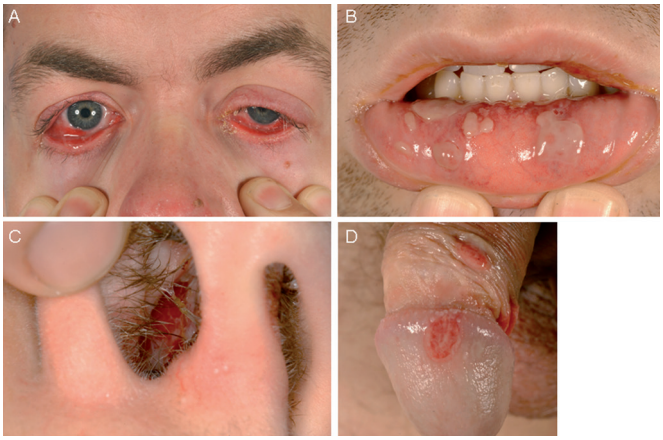

Figure 1: Clinical features in anti-LN-332 MMP patient #1. (A) Conjunctivitis with symblepharon and oedema of the upper eyelid (B), extensive blistering of the oral mucosa (C), erosions on nasal mucosa (D), genital ulcers.

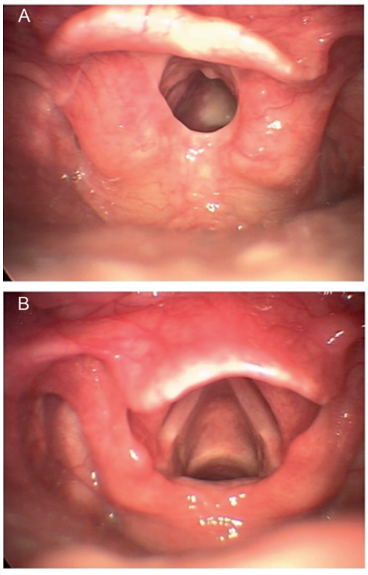

Figure 2: Laryngeal cicatrization of the aryepiglottic folds with supraglottic stenosis in patient #5 (A), and in healthy control. Top is ventral side of patient (B).

Laboratory diagnostics

DIF showed for 9/9 biopsied patients linear IgG deposition along the BMZ in an n-serrated pattern. Complement C3c deposition in the BMZ was present in 7/9 patients (77.8%) and IgA deposition in 2/9 patients (22.2%). IIF of serum on monkey esophagus showed circulating anti-BMZ IgG in 6/10 patient sera (60%). On salt-split skin 9 of the 10 sera (90%) bound to the dermal side of the split. (Table 2)

Immunoblot analysis on keratinocyte extract demonstrated binding to the unprocessed 200-kD and processed 165-kD laminin α3 chain for 5/10 sera (50%). Immunoprecipitation was only performed for two sera and one of these immunoprecipitated LN-332 (50%). ELISA against native LN-332 was positive in 7 of 9 tested sera (77.8%). In patient #3 'knock-out' analysis was positive in type VII collagen deficient skin from a patient with dystrophic epidermolysis bullosa and LN-332 negative skin from patient with Herlitz-type junctional epidermolysis bullosa. In this patient immunoblot analysis revealed IgG reactivity to the 290-kd type VII collagen and to the unprocessed 200-kD and processed 165-kD laminin α3 chain.

Patient	DIF	IIF	SSS	IB	ELISA LN-332	IP	Knock-out	FOAM
1	n-serrated	+	dermal	LN α3	+	np	np	np
2	n-serrated	-	dermal	LN α3	+	np	np	np
3	n-serrated	+	dermal	LN α3 Coll VII	+		Coll VII + LN-332 +	np
4	n-serrated	+	dermal	LN α3	+	np	Coll VII + LN-332 -	+
5	n-serrated	+	dermal	-	+	np	np	np
6	n-serrated	+	dermal	-	-	np	np	np
7	n-serrated	+	dermal	LN α3	-	np	np	np
8	n-serrated	-	dermal	-	+	np	np	np
9	na	-	dermal	-	+	np	np	np
10	n-serrated	-	-	-	np	LN-332	np	np

Table 2: Laboratory diagnostics of 10 patients with anti-LN-332 MMP. DIF: Direct immunofluorescence. (n- or u-serrated pattern); IIF: Indirect immunofluorescence; SSS: Salt-split skin analysis (epidermal or dermal binding); IB: Immunoblot; IP: Immunoprecipitation; na: not available; np not performed

Discussion

In this series of 10 Dutch anti-LN-332 MMP patients we found variable clinical symptoms with more pharyngeal and laryngeal involvement (60%) than is expected in other types of MMP. We added DIF serration pattern analysis to the previously described diagnostic criteria.[18-20] Furthermore we promote aggressive treatment in a multidisciplinary team because of the scarring phenotype of anti-laminin-332 MMP.

Clinical symptoms

Mucosal lesions were seen in 100% of the patients in our series. The oral mucosa and eyes were affected mostly, combined in 50% of all cases. This is in agreement with the results obtained by Egan et al. who described oral mucosa and eyes as predominantly affected with nose, throat and anogenital regions less frequently.[5] The first clinical symptoms like nasal crustae, genital erosions or shortness of breath can mimic other forms of MMP including EBA, and immunopathologic evaluations therefore are of great importance. Wozniak et al. described a case that started with exclusive involvement of the upper respiratory tract that led to progressive dyspnoea and respiratory airway obstruction. After one year oral lesions and scarring conjunctivitis appeared.[26] Skin lesions were seen in 8 of our 10 patients. Five patients had localized blistering and in 3 patients generalized blistering were seen. Skin lesions are not the dominant feature in anti-LN-332 MMP. Two patterns of skin blistering have been described before: (i) generalized bullous pemphigoid-like blistering and (ii) localized Brunsting-Perry-pemphigoid-like vesiculation at the scalp, head, neck and upper trunk.[19]

Scarring of the eyes is a serious aspect of anti-LN-332 MMP. Most of our cases presented with symblepharon and the conjunctivitis/keratitis evoked a burning sensation in the eyes. Coronella et al. described the first case of anti-LN-332 MMP that had only ocular involvement with erythema and oedema of the eyelids but without subconjunctival fibrosis and scarring. This case responded excellent to treatment with methylprednisolone and dapsone.[27] Eye involvement necessitated aggressive treatment to prevent scarring. The emotional consequences of anticipated blindness must not be underestimated. One of our patients committed suicide because of progressive loss of sight of both eyes. In such cases emotional support by a multidisciplinary team is a necessity.

Airway obstruction due to pharyngeal and laryngeal involvement is a serious complication that is seen more in anti-LN-332 MMP than in other forms of MMP.[28, 29] First manifestations are aphonia (loss of voice) due to oedema, erosions and ulcerations of the supraglottic area. This is

followed by scarring of the larynx, and acute upper airway obstruction due to initial laryngeal oedema may occur, necessitating tracheotomy.[5]
Other mucosal surfaces that may be affected include those of the nose, esophagus, genitals and anus.[5,12] Esophageal involvement is described but is most often asymptomatic and probably under diagnosed. When scarring causes stenosis, then dysphagia, phagodynia and gastroesophageal reflux may result. The incidence of esophageal involvement in cicatricial mucous membrane pemphigoid has been estimated to be approximately three percent.[30]

Malignancy

Patients with anti-LN-332 MMP have an increased relative risk for malignancy.[5,13,31,32] Egan et al. described a cohort of 35 patients in which 29% had a solid cancer. The relative risk for malignancy was calculated as 6.8%. Lung cancer and stomach cancer were found mostly.[5] Sadler et al. described 15 anti-LN-332 MMP patients with an associated malignancy. The time between blister onset and cancer diagnosis was 14 months in 12 of the 15 patients. In the other three patients a malignancy was diagnosed several years before or after anti-LN-332 MMP. Adenocarcinoma was found most frequently with five patients having a lung carcinoma.[13] In our study two out of ten patients had an adenocarcinoma of the lung (follow up 8.3 years, range 3-14 years).

Treatment

Patients with anti-LN-332 MMP must be treated promptly and adequately to achieve control of disease and to delay progression. A multidisciplinary approach is a necessity when multiple mucosal sites are affected. In our Centre for Blistering Diseases we have an intense standard cooperation with the ophthalmologist and otolaryngologist for these patients.
Treatment used is always a combination of systemic corticosteroids (prednisolone) and a steroid sparing adjuvant. For acute crisis management, we advise dexamethasone pulse therapy. Azathioprine and cyclophosphamide are preferred choice when ocular involvement is present.[33,34] In our centre azathioprine (3 mg/kg body weight/day) is first choice of therapy as steroid sparing drug. Thiopurine methyltransferase (TPMT) activity must be measured before starting. Therapeutic effect may only be evident after weeks or months. Cyclophosphamide either daily (2 mg/kg body weight/day) or pulsed (750 mg/m² every month intravenous) gave good results. Since cyclophosphamide is a known risk factor for haemorrhagic cystitis and for bladder cancer,

superfluous fluid intake and urine screening is needed. Other immunosuppressant drugs given include dapsone, mycophenolate mofetil, mycophenolic acid, intravenous immunoglobulins and rituximab.

Laboratory diagnostics

Given the disease course and the increased risk of malignancy it is important to distinguish anti-LN-332 MMP from other forms of MMP including the mucosal predominant forms of epidermolysis bullosa acquisita (EBA). In another paper we described how to differentiate EBA from all other subepidermal blistering diseases by DIF serration pattern analysis.[21] Three distinct 'linear' BMZ deposition patterns are recognized: true linear, n-serrated and u-serrated. We speak of a true linear deposition pattern when no serration pattern is present. This is found in mucosa, but can also be present in skin after corticosteroid treatment. The u-serration pattern is found when the autoantibodies bind to type VII collagen as in EBA and bullous systemic lupus erythematosus, including IgA-deposition in IgA-EBA. An n-serrated deposition pattern occurs due to binding of autoantibodies to all other known pemphigoid antigens, the hemidesmosomal BP230, BP180, plectin and LAD-1, and the lamina lucida orientated LN-332 chains and the LN γ1 chain. In our study all patients showed deposition along the BMZ in an n-serrated pattern, thereby excluding EBA (Fig 3)

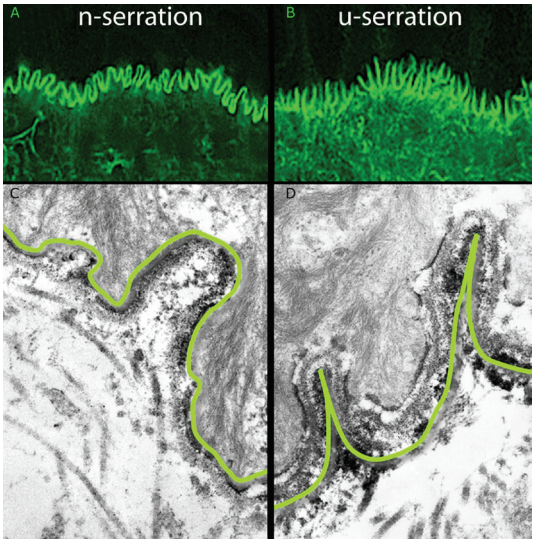

Figure 3: (A+B): Direct immunofluorescence staining of IgG in perilesional skin: (A) n-serration -anti-LN-332 MMP- and (B) u-serration -epidermolysis bullosa acquisita- (C+D): Immuno-electron microscopy IgG: (C) Supra-lamina densa IgG and (D) Sub-lamina densa IgG

The next step is salt-split skin analysis of the serum by indirect IF microscopy. This excludes the MMP forms with hemidesmosomal antigens as BP180. As these are located in the roof of salt-split skin, all sera that bind the roof can be considered not to be anti-LN-332 directed. Of our sera nine bound the floor of the salt-split while one did not bind at all.
The last step is to separate anti-LN-332 MMP from anti-p200 pemphigoid what can be done by immunoblot, ELISA or immunoprecipitation.
In our patients a positive identification of anti-LN α3 antibodies could be made by immunoblot analysis for five out of ten cases (50%). Previous studies have indicated that the α3 subunit is the major antigenic domain in cases of anti-LN-332 MMP [23,35], although others have argued that the reactivity of anti-LN-332 MMP sera is more heterogenous.[36] Fujimoto et al. described a patient with combined IgG binding to the laminin β3 and γ2 chains.[37] Endo et al. described a patient without mucosal involvement and autoantibodies against LN-332 γ2 subunit.[38]
ELISA against native LN-332 was positive in 7/9 cases (77.8 %). Sensitivity and specificity of the LN-332 ELISA for MMP is 75% and 84.3%, respectively.[25]
When omitting the last step, we could diagnose anti-LN-332 MMP or anti-p200 pemphigoid in 9 out of 10 patients by using simple DIF and IIF. Moreover, patient #6, who was negative in ELISA and immunoblot, could be included because of the n-serrated pattern in DIF and circulating IgG autoantibodies that bind to the dermal side of salt-split skin. anti-p200 pemphigoid could formally not be excluded on these laboratory results, but the mucosal dominant clinical presentation makes a diagnosis of anti-LN-332 MMP likely. The clinical presentation of anti-p200 pemphigoid is aspecific and may mimic bullous pemphigoid (most common), linear IgA dermatosis or dermatitis herpetiformis.[39,40]
If no biopsy is available or if serration is absent in the biopsy, the diagnosis has to be established through serum analysis. If immunoblotting, immunoprecipitation or ELISA is not available some valuable information can be obtained through indirect immunofluorescence. First, salt-split skin analysis has to demonstrate that the serum binds to the floor of the split. EBA can then be excluded by fluorescence overlay antigen mapping (FOAM), which is double staining of the serum with monoclonals for respectively type VII collagen and for LN-332.[41,42] Secondly, it is also possible to perform 'knock-out' analysis. This is indirect immunofluorescence analysis on antigen-deficient skin, in this case type VII collagen deficient skin from patients with dystrophic epidermolysis bullosa, and LN-332 deficient skin from patients with Herlitz-type junctional epidermolysis bullosa.[43] In patient #3 'knock-out' analysis was positive in both type VII collagen-deficient and LN-332-deficient skin, while immunoblot analysis also revealed IgG reactivity to the 290-kd type VII collagen and to the unprocessed 200-kD and processed 165-kD laminin

α3 chain. This patient has had a combination of anti-LN-332 MMP and the inflammatory variant of EBA.[24]

Criteria to diagnose anti-LN-332 MMP

The current diagnosis of anti-LN-332 MMP is based on criteria described previously.[18-20] New insights, as described in this article, leads to additional criteria to diagnose anti-LN-332 MMP:

Major criteria:

1) Subepithelial erosions or blisters on mucous membranes frequently associated with scarring phenotype,
2) IgG depositions along the BMZ in the n-serrated pattern by DIF,
3) IgG bound to the dermal side of 1-mol/L NaCL-split human skin by IIF,

Minor criteria:

1) Anti-LN α3, β3, or γ2 IgG binding by immunoblot analysis on keratinocyte cell extract,
2) IgG reactivity to native LN-332 by ELISA,
3) Serum immunoprecipitation of LN-332 trimer,
4) Negative IIF on LN-332 deficient skin, while positive IIF on type VII collagen deficient skin,
5) IgG BMZ deposits overlay LN-332 by fluorescence overlay antigen mapping (FOAM).

To diagnose anti-LN-332 MMP at least three major criteria, or two major criteria and one minor criterion must be obtained.

Conclusion

We describe a new algorithm to diagnose anti-LN-332 MMP. Because of the scarring phenotype, it is important to establish diagnosis in an early stage and to start aggressive treatment in a multidisciplinary team. Because of the increased risk for malignancy patients should be thoroughly screened.

References

1 Domloge-Hultsch N, Gammon WR, Briggaman RA et al. Epiligrin, the major human keratinocyte integrin ligand, is a target in both an acquired autoimmune and an inherited subepidermal blistering skin disease. J Clin Invest 1992; 90: 1628-33.

2 Aumailley M, Bruckner-Tuderman L, Carter WG et al. A simplified laminin nomenclature. Matrix Biol 2005; 24: 326-32.

3 Niessen CM, Hogervorst F, Jaspars LH et al. The alpha 6 beta 4 integrin is a receptor for both laminin and kalinin. Exp Cell Res 1994; 211: 360-7.

4 Rousselle P, Keene DR, Ruggiero F et al. Laminin 5 binds the NC-1 domain of type VII collagen. J Cell Biol 1997; 138: 719-28.

5 Egan CA, Lazarova Z, Darling TN et al. Anti-epiligrin cicatricial pemphigoid: clinical findings, immunopathogenesis, and significant associations. Medicine (Baltimore) 2003; 82: 177-86.

6 Dainichi T, Takeshita H, Moroi Y et al. Cicatricial pemphigoid with autoantibodies against the laminin 5 gamma 2 subunit. Eur J Dermatol 2005; 15: 189-93.

7 Chan LS, Majmudar AA, Tran HH et al. Laminin-6 and laminin-5 are recognized by autoantibodies in a subset of cicatricial pemphigoid. J Invest Dermatol 1997; 108: 848-53.

8 Allbritton JI, Nousari HC, Anhalt GJ. Anti-epiligrin (laminin 5) cicatricial pemphigoid. Br J Dermatol 1997; 137: 992-6.

9 Benoit S, Schmidt E, Sitaru C et al. [Anti-laminin 5 mucous membrane pemphigoid]. J Dtsch Dermatol Ges 2006; 4: 41-4.

10 Hsu RC, Lazarova Z, Lee HG et al. Antiepiligrin cicatricial pemphigoid. J Am Acad Dermatol 2000; 42: 841-4.

11 Lish KM, Washenik K, Yancey KB et al. Anti-epiligrin cicatricial pemphigoid in a patient with HIV. J Am Acad Dermatol 1997; 36: 486-8.

12 Leverkus M, Schmidt E, Lazarova Z et al. Antiepiligrin cicatricial pemphigoid: an underdiagnosed entity within the spectrum of scarring autoimmune subepidermal bullous diseases? Arch Dermatol 1999; 135: 1091-8.

13 Sadler E, Lazarova Z, Sarasombath P et al. A widening perspective regarding the relationship between anti-epiligrin cicatricial pemphigoid and cancer. J Dermatol Sci 2007; 47: 1-7.

14 Fujimoto W, Ishida-Yamamoto A, Hsu R et al. Anti-epiligrin cicatricial pemphigoid: a case associated with gastric carcinoma and features resembling epidermolysis bullosa acquisita. Br J Dermatol 1998; 139: 682-7.

15 Fukushima S, Egawa K, Nishi H et al. Two cases of anti-epiligrin cicatricial pemphigoid with and without associated malignancy. Acta Derm Venereol 2008; 88: 484-7.

16 Gibson GE, Daoud MS, Pittelkow MR. Anti-epiligrin (laminin 5) cicatricial pemphigoid and lung carcinoma: coincidence or association? Br J Dermatol 1997; 137: 780-2.
17 Lenz P, Hsu R, Yee C et al. [Cicatricial pemphigoid with autoantibodies to laminin 5 (epiligrin) in a patient with metastatic endometrial carcinoma]. Hautarzt 1998; 49: 31-5.
18 Yancey KB, Kirtschig G, Yee C et al. Studies of patients with anti-epiligrin cicatricial pemphigoid. J Dermatol 1995; 22: 829-35.
19 Egan CA, Yancey KB. The clinical and immunopathological manifestations of anti-epiligrin cicatricial pemphigoid, a recently defined subepithelial autoimmune blistering disease. Eur J Dermatol 2000; 10: 585-9.
20 Lazarova Z, Sitaru C, Zillikens D et al. Comparative analysis of methods for detection of anti-laminin 5 autoantibodies in patients with anti-epiligrin cicatricial pemphigoid. J Am Acad Dermatol 2004; 51: 886-92.
21 Vodegel RM, Jonkman MF, Pas HH et al. U-serrated immunodeposition pattern differentiates type VII collagen targeting bullous diseases from other subepidermal bullous autoimmune diseases. Br J Dermatol 2004; 151: 112-8.
22 Pas HH, Kloosterhuis GJ, Heeres K et al. Bullous pemphigoid and linear IgA dermatosis sera recognize a similar 120-kDa keratinocyte collagenous glycoprotein with antigenic cross-reactivity to BP180. J Invest Dermatol 1997; 108: 423-9.
23 Kirtschig G, Marinkovich MP, Burgeson RE et al. Anti-basement membrane autoantibodies in patients with anti-epiligrin cicatricial pemphigoid bind the alpha subunit of laminin 5. J Invest Dermatol 1995; 105: 543-8.
24 Jonkman MF, Schuur J, Dijk F et al. Inflammatory variant of epidermolysis bullosa acquisita with IgG autoantibodies against type VII collagen and laminin alpha3. Arch Dermatol 2000; 136: 227-31.
25 Bekou V, Thoma-Uszynski S, Wendler O et al. Detection of laminin 5-specific auto-antibodies in mucous membrane and bullous pemphigoid sera by ELISA. J Invest Dermatol 2005; 124: 732-40.
26 Wozniak K, Waszczykowska E, Hashimoto T et al. Anti-epiligrin cicatricial pemphigoid initially limited to the upper respiratory tract. Br J Dermatol 2006; 154: 779-81.
27 Coronella G, Amato L, Berti S et al. Ocular 'non-scarring' mucous membrane pemphigoid associated with anti-laminin-5 antibodies. Clin Exp Dermatol 2005; 30: 679-81.
28 Whiteside OJ, Martinez Devesa P, Ali I et al. Mucous membrane pemphigoid: nasal and laryngeal manifestations. J Laryngol Otol 2003; 117: 885-8.
29 Shipman AR, Ali I, Murrell DF et al. Mucous membrane pemphigoid: are laminin 5 antibodies a risk factor for laryngeal involvement? J Eur Acad Dermatol Venereol 2009; 23: 169-70.

30 Warren LJ, Wojnarowska F, Wilkinson JD. Oesophageal involvement in cicatricial pemphigoid. Australas J Dermatol 1997; 38: 148-51.
31 Matsushima S, Horiguchi Y, Honda T et al. A case of anti-epiligrin cicatricial pemphigoid associated with lung carcinoma and severe laryngeal stenosis: review of Japanese cases and evaluation of risk for internal malignancy. J Dermatol 2004; 31: 10-5.
32 Egan CA, Lazarova Z, Darling TN et al. Anti-epiligrin cicatricial pemphigoid and relative risk for cancer. Lancet 2001; 357: 1850-1.
33 Dave VK, Vickers CF. Azathioprine in the treatment of muco-cutaneous pemphigoid. Br J Dermatol 1974; 90: 183-6.
34 Brody HJ, Pirozzi DJ. Benign mucous membrane pemphigoid. Response to therapy with cyclophosphamide. Arch Dermatol 1977; 113: 1598-9.
35 Lazarova Z, Yee C, Lazar J et al. IgG autoantibodies in patients with anti-epiligrin cicatricial pemphigoid recognize the G domain of the laminin 5 alpha-subunit. Clin Immunol 2001; 101: 100-5.
36 Hisamatsu Y, Nishiyama T, Amano S et al. Usefulness of immunoblotting using purified laminin 5 in the diagnosis of anti-laminin 5 cicatricial pemphigoid. J Dermatol Sci 2003; 33: 113-9.
37 Fujimoto W, Toi Y, Okazaki F et al. Anti-epiligrin cicatricial pemphigoid with IgG autoantibodies to the beta and gamma subunits of laminin 5. J Am Acad Dermatol 1999; 40: 637-9.
38 Endo Y, Kato M, Kitoh A et al. Pemphigoid without mucosal involvement showing autoantibodies against laminin-332 gamma2 subunit. Br J Dermatol; 163: 1120-2.
39 Dainichi T, Koga H, Tsuji T et al. From anti-p200 pemphigoid to anti-laminin gamma1 pemphigoid. J Dermatol; 37: 231-8.
40 Dilling A, Rose C, Hashimoto T et al. Anti-p200 pemphigoid: a novel autoimmune subepidermal blistering disease. J Dermatol 2007; 34: 1-8.
41 De Jong MC, Bruins S, Heeres K et al. Bullous pemphigoid and epidermolysis bullosa acquisita. Differentiation by fluorescence overlay antigen mapping. Arch Dermatol 1996; 132: 151-7.
42 Wozniak K, Kazama T, Kowalewski C. A practical technique for differentiation of subepidermal bullous diseases: localization of in vivo-bound IgG by laser scanning confocal microscopy. Arch Dermatol 2003; 139: 1007-11.
43 Vodegel RM, Kiss M, Cjm De Jong M et al. The use of skin substrates deficient in basement membrane molecules for the diagnosis of subepidermal autoimmune bullous disease. Eur J Dermatol 1998; 8: 83-5.

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Bullous pemphigoid as pruritus in the elderly; A common presentation

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Abstract

Background

In literature, patients with bullous pemphigoid (BP) have been reported with itch without blisters. Clinical observations in these patients varied from eczematous or urticarial to papular or nodular skin lesions. Here we investigated the spectrum of clinical variants.

Observations

Fifteen patients presented with itch without blisters, but immunopathological findings of BP. Mean age at diagnosis was 81.7 years. No blistering occurred during 2.2 years of follow up. Mean delay of diagnosis was 2.8 years. Clinical symptoms were heterogeneous: pruritus sine materia (no primary skin lesions), eczematous, urticarial, papular and/or nodular skin lesions were seen. Treatment with potent topical corticosteroids or methotrexate sodium led to remission in eleven patients.

Conclusions and Relevance

Itch without skin lesions (pruritus sine materia) can be the only symptom of BP. Therefore it is important to include serological and direct immunofluorescence in the diagnostic algorithm of itch. We propose the unifying term 'pruritic nonbullous pemphigoid' in the spectrum of BP for all patients with immunopathological findings of BP, itch and no blisters.

Introduction

Bullous pemphigoid (BP) is the most common subepidermal autoimmune blistering disease (sAIBD) characterized by autoantibodies targeting the 180-kD bullous pemphigoid antigen (BP180, BPAG2, or type XVII collagen), and/or 230-kD bullous pemphigoid antigen (BP230, BPAG).[1] Elderly are more frequently affected and the associated morbidity is significant. In the recent "Definitions and outcome measures for bullous pemphigoid" pruritus, urticaria and tense blisters were reported as the three main clinical pillars of BP.[2] Diagnosis of BP is commonly based on the combination of clinical presentation, histopathology, direct immunofluorescence (DIF) and the serological detection of autoantibodies by indirect immunofluorescence (IIF) and/or identification of the involved autoantigens.[1-4] Previous studies have described patients with pruritus and immunopathological findings of BP but no blister development.[5-9] As a result, pruritus alone or non-bullous skin lesions are frequently misdiagnosed as xerosis, drug reaction, dermatitis, renal impairment, liver impairment or scabies in elderly patients. In this study we describe a series of patients derived from our biobank database with immunopathological findings of BP who had pruritus sine materia or pruriginous skin lesions without blisters.

Patients meeting the criteria were selected over the period 2002-2012 from the biobank of the Centre for Blistering Diseases in Groningen, the Netherlands. The inclusion criteria were: (i) no blisters, (ii) with either depositions of IgG and/or C3c along the BMZ in the n-serrated or indefinable pattern, or a positive salt-split skin analysis (SSS) with binding to the epidermal side of the blister in combination with a positive NC16A ELISA or a positive BP230 ELISA.
We included one patient (#11) with SSS epidermal binding without any other confirming positive test for BP. This patient was included because of clinical features correlating with pruritic non-bullous pemphigoid and after exclusion of other pruritic disorders (as eczema and drug reactions).
Lesional, perilesional and/or non-lesional skin biopsies were studied by DIF for BMZ depositions of IgG, IgA, IgM and C3c, as described before.[4] Sera of patients were tested by IIF on both monkey esophagus and human salt-split skin substrate.[4]
Immunoblot analysis was performed with extract of cultured human keratinocytes as described before.[3] The anti-NC16A and anti-BP230 ELISA (both from MBL, Nagoya, Japan) were performed according to the protocols of the company. Index values <9 U/mL were considered negative.

Reports of Cases

Fifteen patients met our inclusion criteria, all complained of severe itch. This comprised 12% of all patients diagnosed with BP in our biobank (n=130). Mean age at onset of symptoms was 78.9 years (range 44-93 years). Except for two patients (age 39 and 64 years), all were older than 70 years. Mean age at diagnosis was 81.7 years (range 39-95 years). Mean delay of diagnosis was 2.8 years (range 6 months-11 years). In one case, the patient had pruritic symptoms lasting 11 years that were considered eczema prior to correct diagnosis. The mean follow up time was 2.2 years (range three months-eight years).
Twelve patients had pruritus and excoriations with pruriginous skin lesions comprising eczematous lesions, urticarial plaques, erythematous papules or nodules (Table 1). The distribution varied from the extremities (n=7), localized on trunk (n=2) to generalized (n=3).
Three patients only suffered from pruritus sine materia, in whom linear excoriations were the only visible manifestation of disease (Fig 1). Eleven patients had at least one biopsy with positive DIF that had IgG and/or C3c BMZ deposition. Of seven patients the serration pattern was of the n-type (#1-2, #5, #7, #12, #14-15) while in four cases (#6, #8-9, #13) the pattern was indefinable. DIF was performed on lesional skin (6 out of 7 positive), perilesional skin (3 out of

5 positive), or healthy skin (2 out of 7 positive) (Table 2). In one patient, DIF from perilesional skin was negative, whereas positive from lesional skin. Four patients had negative DIF, but had positive IIF showing IgG circulating autoantibodies on monkey esophagus and SSS (epidermal binding). Immunoblot on BP180 was negative in all but one patients; immunoblot on BP230 was positive in five patients. NC16A ELISA was positive in five patients (range index 13-29 U/mL) and BP230 ELISA in nine patients, range index 16-71 U/mL (Table 2). Routine histology was performed for 12 patients and was either non-specific (#1-2, #4, #8, #11, #14) or showed spongiotic dermatitis with eosinophils (#6-7, #10, #12-13, #15). In all patients treatment was started with potent topical corticosteroid application over the entire body: clobetasol propionate 0.05% cream or momethason furoate 0.1% cream. The dosage of the potent topical corticosteroid was 20 g/d in the first month, 20 g every other day in the second month, 20 g twice a week in the third month, and 20 g once a week in the fourth month. Six of 15 patients reached clinical remission while receiving this treatment. Methotrexate (MTX) at a dose of 5-15 mg weekly in combination with folic acid 5 mg two days after intake was given in six out of nine patients who did not respond on topical corticosteroid therapy (Table 1). Five patients reached clinical remission on this therapy. The sixth patient did not tolerate MTX due to facial edema. Additional treatment with prednisolone 20 mg daily in combination with mycophenolate-mofetil 500 mg once daily also appeared insufficient. Patient 11 died of cardiac failure that was not related to the disease or treatment. Three patients, who did not respond on topical corticosteroid therapy, reached clinical remission on low dose oral prednisolone (5-7.5 mg daily). One of them received additional azathioprine 100 mg/day.

Patient	Sex	Age	Clinical symptoms	Therapy
1	M	87	pruritus sine materia	prednisolone 10 mg + MTX 10 mg
2	M	88	pruritus sine materia	methotrexate 10 mg
3	F	64	pruritus sine materia	clobetasol propionate 0.05% cream
4	F	82	eczematous lesions	clobetasol propionate 0.05% cream
5	M	94	eczematous lesions	clobetasol propionate 0.05% cream
6	M	95	eczematous lesions	prednisolone 7.5 mg + azathioprine 100 mg
7	F	88	eczematous lesions	clobetasol propionate 0.05% cream
8	F	39	eczematous lesions, papules	methotrexate 7.5 mg
9	F	87	papules	clobetasol propionate 0.05% cream
10	F	79	papules	clobetasol propionate 0.05% cream
11	M	94	papules	non response
12	F	92	papules	clobetasol propionate 0.05% cream + methotrexate 5 mg
13	M	71	papules, nodules	methotrexate 15 mg
14	M	87	papules, nodules	momethason furoate 0.1% cream + prednisolone 7.5 mg
15	M	80	urticarial lesions	prednisolone 5 mg

Table 1: Clinical characteristics and therapy of 15 pruritic nonbullous pemphigoid patients

Patient	DIF	Location of biopsy	IIF monkey esophagus, BMZ	IIF SSS, epidermal binding	Immunoblot BP180	Immunoblot BP230	ELISA NC16A (U/mL)	ELISA BP230 U/mL)
1	IgG+	H	IgG+	IgG3+	-	-	-	23
2	IgG+	H	-	-	-	-	-	59
3	-	H	IgG+	IgG2+	-	+/-	29	30
4	-	P, H, L	IgG+	IgG+	-	+/-		25
5	IgA3+ IgG2+ C3c2+	H	-	IgA+	-	-	-	-
6	IgG+/2+	L	-	-	-	-	17	-
7	IgG2+ C3c2+	L	IgG+	IgG+	+	+	27	71
8	IgM, IgG+	L	IgG+	IgG+	-	-	-	-
9	IgA+IgG+ C3c2+	L	IgG+	-	-	-	-	-
10	-	P, H	IgG+	IgG+	-	-	23	19
11	-	P, H	IgG+	IgG2+	-	-	-	-
12	IgG+	L	IgG+	IgG2+	-	+	-	34
13	C3c 2+	L	IgG+	IgG2+	-	+/-	13	38
14	IgG+	P		-	-	-	-	-
15	IgG+ C3c+	P	IgG+	IgG3+	-	-	-	16

Table 2: Immunodermatological findings in 15 pruritic nonbullous pemphigoid patients.

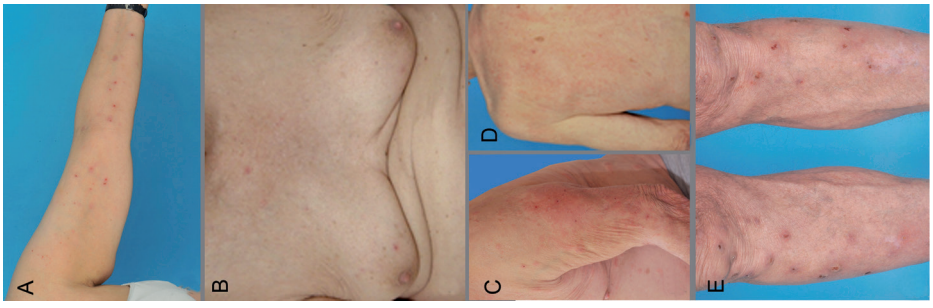

Figure 1: Clinical presentations of pruritic nonbullous pemphigoid by (A) pruritus sine materia (patient 2), (B) urticarial plaques (patient 15), (C) eczematous lesions (patient 6), and (D) excoriated nodules (patient 13)

Comment

BP may start with pruritus in the prodromal stage, while blisters develop weeks or months later.[1,10-14] In a Swiss study population 20% of 160 diagnosed BP patients presented without blisters.[15] In the literature patients have been reported with itch combined with immunopathological findings compatible with BP but without the formation of blisters, when followed up for several months or even years.[5-9,11,14,16-19] In previous studies most of these patients were elderly with an age above 70 years (follow up four months- six years). In the present study mean age was 81.7 years. According to the definitions from an international expert panel, the symptom pruritus is considered to be sufficient for the diagnosis of BP, if the immunological criteria are met.[2]

Pruritus is also component of the subjective Bullous Pemphigoid Disease Area Index (BPDAI). Among all patients with pemphigoid in our Centre for Blistering Diseases about one in eight did not develop blisters, while all of them experienced itch. The skin symptoms consisted of erythematous, excoriated papules or nodules and urticarial or eczematous plaques. Three out of 15 patients (20%) only had pruritus without primary skin lesions (pruritus sine materia). This implies that a dermatologist who sees an elderly patient with unexplained itch, even without evident skin manifestations, should think of BP. In such case one should perform both IIF and DIF. Diagnosis was reached by a positive DIF with IgG and/or C3c along the epidermal BMZ, or SSS-epidermal binding in combination with a positive ELISA (NC16A or BP230). Earlier studies reported individual cases suggestive of BP in the absence of a positive DIF.[5,20] Additional findings included a positive IIF on monkey esophagus or detection of BP180 or BP230 autoantibodies by immunoblot. Our ELISA results concord with Feliciani et al. who found that BP230 ELISA is more often positive than NC16A ELISA.[20] In our study we found more positive DIF results from biopsies of lesional skin than those of perilesional or healthy skin. In one case perilesional DIF from healthy skin was negative while lesional DIF from a papule was positive. Future studies should point out if a lesional DIF biopsy for this group of patients is indicated, instead of perilesional which is recommended for BP.[21] Histopathology is either nonspecific or shows spongiotic dermatitis with eosinophils, which can be suggestive for BP.[5-7,17]

In the literature there is no unanimity on how to name this subset of patients. The coined terms include 'pruritic pemphigoid', 'pemphigoid nodularis', 'papular pemphigoid', 'prurigo-nodularis like pemphigoid', 'nonbullous BP', 'prodromal BP' and 'BP incipiens'.[5-7,9,20,22,23] In our opinion the latter two can be used only retrospectively when blisters have appeared. Furthermore, lesions that may accompany the pruritus were heterogeneous. The term nonbullous pemphigoid seems adequate, but lacks the most important clinical characteristic: pruritus. We therefore propose the

unifying term 'pruritic nonbullous pemphigoid', to trigger the dermatologist to think of pemphigoid when confronted with an elderly who complains of itch.

Treatment of this intense pruritic condition is essential. In line with previous authors we suggest a therapeutic ladder starting with whole body application of potent topical corticosteroids as clobetasol propionate cream.[6,20,24] If non-responding, our preferred treatment is MTX in a low-dose prescription (5-15 mg a week) corresponding with treatment of BP.[25-27] When insufficient a wide range of other therapeutic options have proven successful in the past.[5-9,17,18,20]

Progression of pruritic nonbullous pemphigoid to bullous pemphigoid rarely occurs.[6,28] The most intriguing question is why these patients do not develop blisters. When antigens were identified it was mostly BP230.[29,30] Rabbit and mouse model studies showed that BP230 autoantibodies induce skin fragility but do not lead to development of blisters. Another explanation might be that IgE autoantibodies against BP180,[31] induce pruritus, while IgG along the BMZ is insufficient to induce the blister.[32]

References

1 Di Zenzo G, Marazza G, Borradori L. Bullous pemphigoid: physiopathology, clinical features and management. Adv Dermatol 2007;23:257-288.

2 Murrell DF, Daniel BS, Joly P, Borradori L, Amagai M, Hashimoto T, et al. Definitions and outcome measures for bullous pemphigoid: Recommendations by an international panel of experts. J Am Acad Dermatol 2012; 66 (3):479-485.

3 Pas HH. Immunoblot assay in differential diagnosis of autoimmune blistering skin diseases. Clin Dermatol 2001 Sep-Oct;19(5):622-630.

4 Vodegel RM, Jonkman MF, Pas HH, de Jong MC. U-serrated immunodeposition pattern differentiates type VII collagen targeting bullous diseases from other subepidermal bullous autoimmune diseases. Br J Dermatol 2004 Jul;151(1):112-118.

5 Alonso-Llamazares J, Rogers RS,3rd, Oursler JR, Calobrisi SD. Bullous pemphigoid presenting as generalized pruritus: observations in six patients. Int J Dermatol 1998 Jul;37(7):508-514.

6 Lamb PM, Abell E, Tharp M, Frye R, Deng JS. Prodromal bullous pemphigoid. Int J Dermatol 2006 Mar;45(3):209-214.

7 Safa G, Darrieux L. Nonbullous pemphigoid treated with doxycycline monotherapy: report of 4 cases. J Am Acad Dermatol 2011 Jun;64(6):e116-8.

8 Wever S, Rank C, Hornschuh B, Hashimoto T, Nishikawa T, Brocker EB, et al. Bullous pemphigoid simulation subacute simple prurigo. Hautarzt 1995 Nov;46(11):789-795.

9 Wolf R, Ophir J, Dechner E. Nonbullous bullous pemphigoid. Int J Dermatol 1992 Jul;31(7):498-500.

10 Parker SR, MacKelfresh J. Autoimmune blistering diseases in the elderly. Clin Dermatol 2011 Jan-Feb;29(1):69-79.

11 Amato DA, Silverstein J, Zitelli J. The prodrome of bullous pemphigoid. Int J Dermatol 1988 Oct;27(8):560-563.

12 Asbrink E, Hovmark A. Clinical variations in bullous pemphigoid with respect to early symptoms. Acta Derm Venereol 1981;61(5):417-421.

13 Tamada Y, Yokochi K, Oshitani Y, Nitta Y, Ikeya T, Hara K, et al. Pemphigoid nodularis: a case with 230 kDa hemidesmosomes antigen associated with bullous pemphigoid antigen. J Dermatol 1995 Mar;22(3):201-204.

14 Barker DJ. Generalized pruritus as the presenting feature of bullous pemphigoid. Br J Dermatol 1983 Aug;109(2):237-239.

15 Di Zenzo G, Della Torre R, Zambruno G, Borradori L. Bullous pemphigoid: from the clinic to the bench. Clin Dermatol 2012 Jan-Feb;30(1):3-16.

16 Ross JS, McKee PH, Smith NP, Shimizu H, Griffiths WA, Bhogal BS, et al. Unusual variants of pemphigoid: from pruritus to pemphigoid nodularis. J Cutan Pathol 1992 Jun;19(3):212-216.
17 Strohal R, Rappersberger K, Pehamberger H, Wolff K. Nonbullous pemphigoid: prodrome of bullous pemphigoid or a distinct pemphigoid variant? J Am Acad Dermatol 1993 Aug;29(2 Pt 2):293-299.
18 Powell AM, Albert S, Gratian MJ, Bittencourt R, Bhogal BS, Black MM. Pemphigoid nodularis (non-bullous): a clinicopathological study of five cases. Br J Dermatol 2002 Aug;147(2):343-349.
19 Schmidt E, Sitaru C, Schubert B, Wesselmann U, Kromminga A, Brocker EB, et al. Subacute prurigo variant of bullous pemphigoid: autoantibodies show the same specificity compared with classic bullous pemphigoid. J Am Acad Dermatol 2002 Jul;47(1):133-136.
20 Feliciani C, Caldarola G, Kneisel A, Podstawa E, Pfutze M, Pfutzner W, et al. IgG autoantibody reactivity against bullous pemphigoid (BP) 180 and BP230 in elderly patients with pruritic dermatoses. Br J Dermatol 2009 Aug;161(2):306-312.
21 Anstey A, Venning V, Wojnarowska F, Bhogal B, Black MM. Determination of the optimum site for diagnostic biopsy for direct immunofluorescence in bullous pemphigoid. Clin Exp Dermatol 1990 Nov;15(6):438-441.
22 Massa MC, Connolly SM. Bullous pemphigoid with features of prurigo nodularis. Arch Dermatol 1982 Nov;118(11):937-939.
23 Tashiro H, Arai H, Hashimoto T, Takezaki S, Kawana S. Pemphigoid nodularis: two case studies and analysis of autoantibodies before and after the development of generalized blistering. J Nihon Med Sch 2005 Feb;72(1):60-65.
24 Joly P, Fontaine J, Roujeau JC. The role of topical corticosteroids in bullous pemphigoid in the elderly. Drugs Aging 2005;22(7):571-576.
25 Dereure O, Bessis D, Guillot B, Guilhou JJ. Treatment of bullous pemphigoid by low-dose methotrexate associated with short-term potent topical steroids: an open prospective study of 18 cases. Arch Dermatol 2002 Sep;138(9):1255-1256.
26 Du-Thanh A, Merlet S, Maillard H, Bernard P, Joly P, Esteve E, et al. Combined treatment with low-dose methotrexate and initial short-term superpotent topical steroids in bullous pemphigoid: An open, multicenter, retrospective study. Br J Dermatol 2011;165(6):1337-1343.
27 Paul MA, Jorizzo JL, Fleischer AB,Jr, White WL. Low-dose methotrexate treatment in elderly patients with bullous pemphigoid. J Am Acad Dermatol 1994 Oct;31(4):620-625.
28 Nakatani T, Inaoki M, Takehara K. Bullous pemphigoid with a prolonged prodrome. J Dermatol 2008 Jul;35(7):433-436.

29 Hall RP,3rd, Murray JC, McCord MM, Rico MJ, Streilein RD. Rabbits immunized with a peptide encoded for by the 230-kD bullous pemphigoid antigen cDNA develop an enhanced inflammatory response to UVB irradiation: a potential animal model for bullous pemphigoid. J Invest Dermatol 1993 Jul;101(1):9-14.

30 Guo L, Degenstein L, Dowling J, Yu QC, Wollmann R, Perman B, et al. Gene targeting of BPAG1: abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degeneration. Cell 1995 Apr 21;81(2):233-243.

31 Dimson OG, Giudice GJ, Fu CL, Van den Bergh F, Warren SJ, Janson MM, et al. Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. J Invest Dermatol 2003 May;120(5):784-788.

32 Fania L, Caldarola G, Muller R, Brandt O, Pellicano R, Feliciani C, et al. IgE recognition of bullous pemphigoid (BP)180 and BP230 in BP patients and elderly individuals with pruritic dermatoses. Clin Immunol 2012 Jun;143(3):236-245.

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Whole body application of a potent topical corticosteroid for bullous pemphigoid

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Abstract

Background Current standard of treatment of bullous pemphigoid (BP) is systemic oral corticosteroids (CS). However significant iatrogenic morbidity and mortality is reported. Studies have shown that topical potent CS is safer than oral prednisolone in BP.

Objectives To examine the local and systemic efficacy and adverse effects of whole body clobetasol propionate cream application in patients with mild or severe BP.

Methods Open, clinical records-based retrospective analysis of a series of mild (n=40) and severe (n=34) BP patients, treated with ranging doses (20-40g/day) clobetasol propionate cream. For assessing systemic effects we observed in selected cases eosinophil count and morning urine cortisol level.

Results Patients with mild BP achieved in 90.0% disease control and in severe BP in 73.5%. Complete remission was achieved in mild BP in 64.1% (35.9% off therapy and 28.2% on therapy) versus 41.2% in severe BP (5.9% off therapy and 35.3% on therapy). Local adverse effects were mainly skin atrophy (14.9%) and purpura (5.4%). Systemic adverse effects were rare (n=3) and consisted of deep vein thrombosis, hypertrichosis and adrenocortical insufficiency. Systemic effect was witnessed by immediate drop of eosinophil count, and decrease in the morning urine cortisol in selected cases.

Conclusions Topical whole body application of clobetasol propionate cream as monotherapy can be effective and safe in the induction phase of treatment in mild BP and severe BP. When relapse occurs adjuvant systemic medication is mandatory. Potent CS works locally and systemically against BP, at the price of significant local and less significant systemic adverse effects.

Introduction

Bullous pemphigoid (BP) is an acquired subepidermal autoimmune blistering disease (AIBD) preferentially in elderly characterized by pruritic eruption of bullae, urticaria, erythema or papules.[1,2] The targets of the autoantibodies are 180-kD bullous pemphigoid antigen (BP180, BPAG2, or type XVII collagen), and/or 230-kD bullous pemphigoid antigen (BP230, BPAG1).[3,4] These are components of the hemidesmosomal plaque, adhesion structures that anchor the basal cells to the underlying epidermal basement membrane zone (EBMZ). Patients with BP almost always present with itch and some with urticarial plaques that may evolve into vesicles and blisters.[5] Treatment of BP is challenging because of the older age of the patients, and the comorbidities like neurological and cardiovascular disorders that are associated with the age and the disease.[1] Multiple drugs are often used, whereas these patients are at high risk of

adverse drug reactions and side-effects.[1,2,6-8] Treatment can be divided into two groups: i) topical application of potent corticosteroids (CS) -clobetasol propionate cream- or ii) systemic oral CS with or without steroid sparing immunosuppressive drug. Systemic oral CS is the best established treatment according to current evidence,[5] but iatrogenic morbidity and mortality is reported. Potent topical CS should be considered in any patient with BP.[9]

Recently, French colleagues assembled in the 'Groupe Bulle' conducted two randomized controlled trials in BP, and showed that topical clobetasol propionate cream application on the whole body, progressively tapered over 12 or four months, were both effective while reducing the morbidity in severe BP due to treatment.[6,10]

It is likely that the high efficacy of whole body topical clobetasol propionate application is due to both local and systemic effects. However systemic oral CS raised more adverse effects (54%) than topical clobetasol (29%).[6]

In this study we examined the efficacy and adverse effects of whole body topical clobetasol propionate cream application in patients with mild or severe BP. Moreover we investigated cortisol levels and eosinophil counts in selected cases and found systemic effects using a potent topical CS.

Material and Methods

Patients

Potent topical CS therapy started in 2002 at the Centre for Blistering Diseases in Groningen after the publication of Joly et al.[6] For the current study, patients with diagnosis of bullous pemphigoid were selected from our biobank over the period 2002-2010 using the following criteria: i) subepidermal blistering and/or erosions of the skin, and ii) DIF showing deposits of IgG along the EBMZ in the n-serrated pattern, and/or iii) autoantibodies binding to BP180 and/or BP230 either by immunoblot or NC16A-ELISA. All files of BP patients in this period were examined and those patients that had received potent topical CS were included. In case of missing data patients were excluded.

Patients were divided into two groups: i) mild BP and ii) severe BP, and were compared for the local and systemic efficacy and adverse effects of clobetasol propionate cream. This selection was determined on the number of bullae that were seen at the day of the first consultation. Mild BP was defined by <10 bullae and severe BP by ≥10 bullae according to Joly et al.[6] Patients with mild BP were treated with an initial ranging dose of 20-40 g/day clobetasol propionate cream. Patients with severe BP were also treated with an initial ranging initial dose of

20-40 g/day clobetasol propionate cream (Table 1). The clobetasol propionate cream was tapered in four months according to the following protocol: first month clobetasol propionate cream every day, second month every other day, third month twice a week and fourth month once a week.
Osteoporosis prophylaxis or antacids were not routinely given. Few patients who received steroid sparing immunosuppressive medication besides potent topical CS were also included. Adjuvant medication was started after disease control when after a relapse potent topical CS was not enough to control the disease.

Primary outcomes

Outcome measures as defined by the recent international consensus were used.[5] Primary outcomes were: disease control and remission. Disease control was defined as: the time interval from baseline to the time at which new lesions cease to form and established lesions begin to heal or pruritic symptoms start to abate. Remission was divided into three groups: i) complete remission (CR) off therapy, ii) CR on therapy and iii) partial remission (PR). CR off therapy was defined as the absence of new or established lesions or pruritus while the patient is off all BP therapy for at least two months; CR on therapy as the absence of new or established lesions or pruritus while the patient is receiving minimal therapy (less than 10mg prednisolone and/or adjuvant), not for at least 2 months and PR is defined as the presence of transient new lesions that heal within one week without treatment or pruritus less than once per week.

Secondary outcomes

Secondary outcomes were relapse, adverse effects, eosinophil count and pharmaco-economics. A relapse was defined as the appearance of three or more new lesions a month (blisters, eczematous lesions or urticarial plaques) or at least one large (>10cm diameter) eczematous lesion or urticarial plaques that do not heal within one week, or the extension of established lesions or daily pruritus in a patient who has achieved disease control. In patients who had a relapse during the period when the dose was being reduced, the dose was increased to the previous level that had permitted control of the disease. Adverse effects were divided into local (like skin atrophy, striae or purpura and others) and systemic adverse effects (like adrenocortical insufficiency, diabetes mellitus, cardiovascular and neurovascular disorders or infections and others). Eosinophil count was determined at the start of the treatment (in 10^9/l) and was measured within the

next 12 days in nine selected cases. These patients did not receive any other medication than topical potent CS. Medication costs were calculated by sum up the costs of daily clobetasol propionate cream treatment in mild and severe BP patients. These results were compared with the costs of oral CS, osteoporosis prophylaxis and antacids treatment in mild and severe BP patients.

Morning urine cortisol (nmol/l)

In three patients morning urine cortisol (nmol/l) was measured. The first patient received a treatment of 30 g triamcinolone acetonide 0.1% cream on day 1-3 on their whole body, and 30 g clobetasol propionate on day 4-6 cream on their whole body. On day 1, 3 and 6 the morning urine cortisol (nmol/l) was measured. Two other patients with BP received a treatment of 20 g clobetasol propionate on 6 consecutive days. On day 1, 3 and 6 the morning urine cortisol (nmol/l) was measured.

Statistical analysis

Distributions of survival for disease control, remission and relapse were calculated, the Kaplan-Meier method for both disease severity groups, and statistically compared by log-rank test. Beforehand the two disease severity groups were compared for age, and dosage of clobetasol propionate cream using the rank sum test. Occurrence of itch, urticarial plaques, frequency of adverse effects, and the use of other medication were compared by the Fisher exact test. Use of other immunosuppressive medication was adjusted by a Cox-model to adjust for suspected a priori prognostic significance. For all tests, two-sided p- values of less than 0.05 were considered to indicate statistical significance.

Results

Patients

From a cohort of 135 BP patients in the time frame of 11 years, 115 patients were treated with whole body potent topical CS. Because of missing data finally 74 patients were eligible for further analysis. Of the remaining 74 patients, 40 patients had less than 10 bullae (mild BP) and 34 patients had 10 or more bullae (severe BP) initially (Fig 1). Baseline characteristics of the patients are shown in table 2. Both groups were comparable for gender (mild group: 27.5% males; severe group: 38.2% males) and mean age (mild group: 71.4 years; severe group: 68.2

years). The follow up time was 1.5 weeks - 7.5 years (median 38 weeks). In the mild BP group (n=40), eight patients were in poor conditions as in dependent of nursing. Fifteen of the 40 patients had associated neurological disorders like cerebrovascular accident (n=10), multiple sclerosis (n=1), parkinson's disease (n=1) and epilepsy (n=3). In the severe BP group (n=36), 11 were in poor conditions as in dependent of nursing. Seven of the 36 patients had associated neurological disorders like cerebrovascular accident (n=3), multiple sclerosis (n=1), limb girdle muscular dystrophy (n=1) and alzheimer's disease (n=2).
Overall both groups were treated with a comparable dose of clobetasol propionate cream, with a mean starting dose of 24.3 g in the mild group and 25.3 g in the severe group, although in both groups ranging doses in single patients were used (Table 1) In the severe group, more patients used adjuvant medication for BP (82.4%) than patients in the mild group (50.0%) (Table 2) However adjuvant medication was started after disease control when after a relapse potent topical CS was not enough to control the disease. Three patients (4.1%) died during potent topical CS treatment due to detoriation of the general condition (n=2), and acute lymphoblastic leukemia (n=1). The three diseased patients were excluded from the survival analysis of remission and relapse.

	20g/day	30g/day	40g/day
Mild BP (40 patients) < 10 bullae*	31 patients	1 patient	8 patients
Severe BP (36 patients) ≥ 10 bullae*	19 patients	9 patients	6 patients

Table 1: Initial starting dose of clobetasol propionate cream therapy in single patients. The following protocol was used: first month clobetasol propionate cream every day, second month every other day, third month twice a week and fourth month once a week. *: the number of blisters seen at the day of first consultation

Disease control, remission and relapse

Control of disease activity was achieved in 36 of the 40 patients with mild BP (90%) and in 25 of the 34 patients with severe BP (73.6%) (P=0.155) (Fig 2). Four patients in the mild BP group, who did not achieve control of disease activity used initially 20g/day clobetasol propionate cream (P= 0,708). Of the nine patients in the severe BP group who did not achieve control of disease activity, three patients used 20g/day clobetasol propionate cream, three patients used

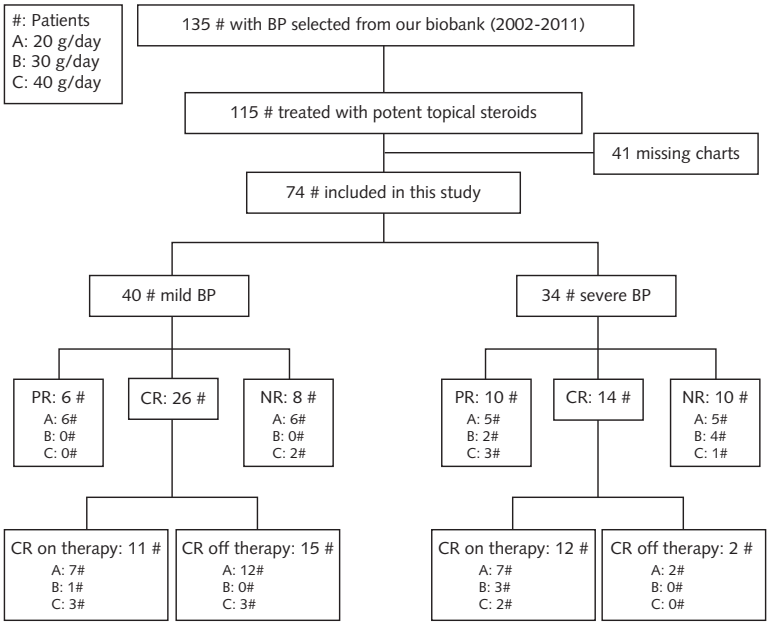

Figure 1: Flowchart of the study. BP: bullous pemphigoid; CR: complete remission; PR: partial remission; NR: no remission.

30g/day clobetasol propionate cream and three patients used 40g/day clobetasol propionate cream initially (P= 0,279). The median time to achieve disease control was 20 days. In the mild group 64.1% (n=26) reached CR: 35.9% off therapy (n=15) and 28.2% while on therapy (n=11). PR was seen in 15.4% (n=6), whereas 20.5% (n=8) of the patients reached no remission. In the severe group 41.2% reached CR (n=14): 5.9% off therapy (n=2) and 35.3% on therapy (n=12). PR was reached in 29.4% of the patients (n=10), and 29.4% reached no remission (n=20). The different initial doses clobetasol propionate cream a day, PR, CR on and off therapy and no remission in every single patient is depicted in figure 1. Complete remission was more achieved in the mild BP group (P= 0.025). However no significant difference between the two groups exists for reaching any remission (P= 0.278).

Of those with an initial control of disease, 55.7% experienced a relapse, with 55.6% in the mild group and 56.0% in the severe group (P= 0.814). The median time until the occurrence of a relapse is 19 weeks, so the relapses occurred mostly after stopping treatment.

Local and systemic adverse effects

Overall, 23 adverse effects were reported in 74 patients, 17.5% of the patients in the mild group and 29.4% in the severe group experienced one or more adverse effects (P = 0.274) with a follow up of 7 years (Table 2).

Main adverse effects in the 74 patients were local like skin atrophy (n=13), purpura (n=4), striae (n=2) and xerosis cutis (n=1). Systemic adverse effects were reported in only three patients: deep vein thrombosis (n=1), adrenocortical insufficiency (n=1) and hypertrichosis (n=1).

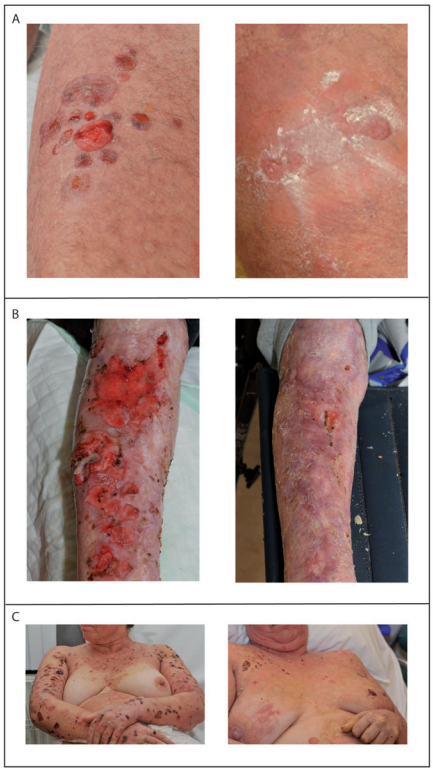

Figure 2: Clinical features patients treated with potent topical corticosteroid therapy: patient (A) treatment initially with 20g/day clobetasol propionate cream and result after 1 week; patient (B) treatment initially with 30g/day clobetasol propionate cream and result after 2 months; patient (C): treatment initially with 30g/day clobetasol propionate cream and result after 4 months

		All BP patients (N=74)	Mild BP (N=40)	Severe BP (N=34)	P value
Male		24 (32.4%)	11 (27.5%)	13 (38.2%)	0,455
Mean Age ± SD		69.9 ± 16.4	71.4 ± 14.4	68.2 ± 18.5	0.402
Mean number of blisters ± SD		18.8 ± 23,2	3.6 ± 3,4	36.7 ± 23,9	0.000
Itch		73 (98.6%)	39 (97.5%)	34 (100%)	1.000
Urticaria		21 (28.4%)	6 (15.0%)	15 (44.1%)	0.009
Mean begin doses clobetasol (g) ± SD		24.7 ± 8.0	24.3 ± 8.1	25.3 ± 7.9	0.578
Use of other medication		48 (64.9%)	20 (50.0%)	28 (82.4%)	0.007
	azathioprine	6 (8.1%)	4 (10.0%)	2 (5.9%)	
	doxycycline, nicotinamide	11 (14.9%)	7 (17.5%)	4 (11.8%)	
	prednisolone	11 (14.9%)	4 (10.0%)	7 (20.6%)	
	methotrexate	1 (1.4%)	1 (2.5%)	0 (0%)	
	azathioprine, prednisolone	13 (17.6%)	2 (5.0%)	11 (32.8%)	
	doxycycline, nicotinamide, prednisolone	5 (6.8%)	2 (5.0%)	3 (8.8%)	
	azathioprine, doxycycline, nicotinamide, prednisolone	1 (1.4%)	0 (0%)	1 (2.9%)	
Adverse effects		17 patients (23.0%) 23	7 patients (17.5%) 8	10 patients (29.4%) 15	0.274
	atrophy	13	6	7	
	purpura	4	2	2	
	striae	2	0	2	
	xerosis	1	0	1	
	deep vein thrombosis	1	0	1	
	adrenocortical insufficiency	1	0	1	
	hypertrichosis	1	0	1	

Table 2: Patient characteristics.

Systemic effect

A decrease in number of eosinophils was seen in nine patients after the application of clobetasol propionate cream. This effect was already observed after one day of the start of treatment. Eosinophil number dropped to normal range (0.0-0.40 10^9/L) after an average of 5.6 days (Fig 3).

In one patient morning urine cortisol did not drop after three days of 30 g triamcinolon acetonate 0.1% cream applied on the whole body. In contrast, after 30 g clobetasol propionate cream on the same area, morning urine cortisol dropped to 5.0 nmol/L (Fig 4). This patient did not use any other medication. A second patient applied 20 g clobetasol propionate cream on the whole body for 6 consecutive days. Morning urine cortisol dropped to 2.0 nmol/L. A third patient applied 20 g clobetasol propionate cream on the whole body for 6 consecutive days. Morning urine cortisol dropped from 14.4 nmol/L (day 1) to 3.4 nmol/L (day 6). Both patients did not use any other medication. These results demonstrate systemic effects of whole body potent CS application.

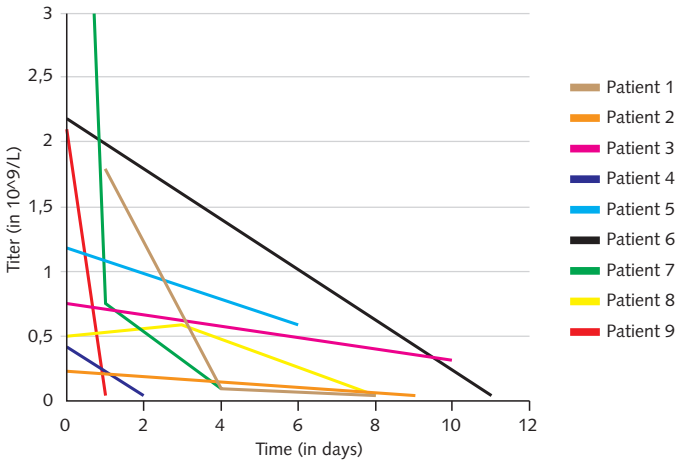

Figure 3: Number of eosinophils after treatment with potent topical corticosteroid therapy

Pharmaco-economics

In the Netherlands the costs of 100 gram clobetasol propionate cream are €11.50 ($14.77). For a patient treated with the 20g/day clobetasol propionate cream protocol the costs for the

treatment of one patient with potent topical CS during 4 months will be €124.20 ($159.56) and for patients treated with 30g/day €174.80 ($224.57). The costs of prednisolone (20 mg) is €0.14 ($0.18), osteoporosis prophylaxis costs €0.38 ($0.49) per day, and antacids costs €0.02 ($0.03) per day. A mild BP patient will be treated with 20 gram prednisolone per day tapered in 3 months in combination with osteoporosis prophylaxis and antacids. Total cost will be approximate €40.46 ($52.30)

A severe BP patient will be treated with 40 gram prednisolone per day tapered in 3 months in combination with osteoporosis prophylaxis and antacids. Total cost will be approximate €47.46 ($61.35)

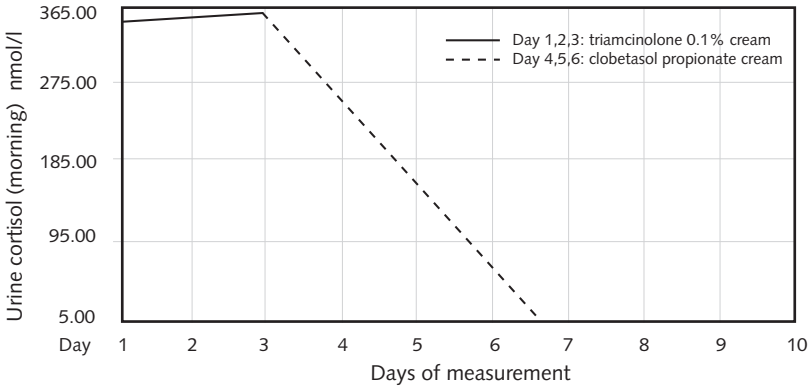

Figure 4: Morning urine cortisol measurement. Day 1-3: whole body application of 30 g triamcinolon acetonate 0.1% cream. Day 4-6 whole body application of 30 g clobetasol propionate cream.

Discussion

Treatment of BP is challenging because of the older age of the patients and their associated comorbidities. Joly et al. reported that topical application of clobetasol propionate cream on the whole body has a similar efficacy in severe BP as oral CS with disease control within 12 months of >98%, at the cost of much lower morbidity and mortality (50% reduction).[6,10] In our study we show a high disease control (90%) in mild BP after four months with topical potent CS close to that previously reported.[10] The rate of disease control in severe BP was lower (73.6%) than reported by Joly et al. (90%), but our initial mean starting dose (25.3g/day) was lower than in

the study by Joly et al. (from 30-40g/day).[10] Our results confirm that whole body application of clobetasol propionate as monotherapy is sufficient to control disease in mild BP and in severe BP. The overall occurrence of a relapse was similar compared with the study of Joly et al. in mild BP (56% versus 43%), and in severe BP (56 % versus 35%) using the four months regimen. These relapse rates compares favorably with relapse rates obtained with systemic CS (60 to 80%).[6,10] Because these relapses were mainly observed after stopping treatment at four months, adjuvant maintenance therapy may be useful to keep the disease in remission after the induction phase with clobetasol. We prefer azathioprine in 2-3 mg/kg/day dosage. Other adjuvant options are methotrexate, dapsone, mycophenolate mofetil, mycophenolic acid, cyclophosphamide, intravenous immunoglobuline and rituximab. Recently the "Groupe Bulle" reported that the combination of initial short-term potent topical steroids (clobetasol propionate and bethamethasone propionate) with low-dose methotrexate resulted in an even better result.[11]

Tolerance of treatment is a crucial point in fragile, especially in elderly BP patients with significant, often numerous comorbidities and associated neurological disorders. In the French studies side effects were observed in 29% of patients treated with clobetasol propionate cream, while in our series this was 23%. The experienced side effects were related to clobetasol treatment and mostly local such as skin atrophy or purpura, and did not require treatment discontinuation. Systemic adverse effects were only seen in three patients with severe BP (hypertrichosis, deep vein thrombosis, adrenocortical insufficiency) and none were observed in mild BP. This low percentage of systemic adverse events demonstrates the safety of topical whole body CS. Furthermore it is important to have attention for the pharmaco-economics. In regard to medication costs, application of topical CS is three times more expensive compared to treatment with oral CS in BP, although the treatment is cheap and do does not extend above €175 in 4 months. The costs for whole body application by home care nurses were not accounted for, and substantially increase the total costs. Moreover, lubricating the whole body can be stressful for elderly patients. However, the costs due to more adverse systemic events by oral CS may push the financial balance back in favour of topical CS.

The whole body application of clobetasol propionate cream has local and systemic effects witnessed by the drop in morning urine cortisol and eosinophil count. In these patients no systemic side effects were seen. A validated assay for percutaneous absorption of clobetasol was not available in our study period and became available in 2010 by Van Velsen et al.[12] The same authors have showed the systemic absorption and effects of whole body application of clobetasol propionate 0.05% cream in patients with atopic dermatitis. A single application resulted in detectable serum levels of clobetasol propionate of 0.112-4.504 ng/ml, and decreased

cortisol levels, from 0.47 ± 0.18 µmol/l to 0.04 ± 0.05 µmol/l after 1 day.[13] Moreover the same whole body application of a less potent topical CS (betamethason valerate) did show in a few patients systemic absorption and fluticasone propionate cream application did not show systemic absorption.

More studies are needed to evaluate the systemic effects and the serum levels of clobetasol propionate after whole body topical clobetasol application in BP patients. Limitations of our study are in first place the differences in starting dose of clobetasol propionate cream in single patients. Furthermore some patients received also immunosuppressants, although these medications in all cases were started after the fourth month regimen when a patient received a relapse. Secondly we have a high lost of patients (n=41) because of missing data. These limitations can impair the results of our study.

In conclusion we found that whole body application of clobetasol propionate cream is effective in the induction phase of treatment in patients with mild BP and in patients with severe BP. We advice to add an immunosuppressive drug in BP patients with a relapse when topical clobetasol propionate cream is not sufficient anymore. The whole body clobetasol therapy is safe and may be better tolerated than systemic oral CS.

References

1 Bastuji-Garin S, Joly P, Lemordant P et al. Risk factors for bullous pemphigoid in the elderly: a prospective case-control study. J Invest Dermatol 2011; 131: 637-43.

2 Langan SM, Smeeth L, Hubbard R et al. Bullous pemphigoid and pemphigus vulgaris-incidence and mortality in the UK: population based cohort study. Bmj 2008; 337: a180.

3 Bernard P, Vaillant L, Labeille B et al. Incidence and distribution of subepidermal autoimmune bullous skin diseases in three French regions. Bullous Diseases French Study Group. Arch Dermatol 1995; 131: 48-52.

4 Zillikens D, Wever S, Roth A et al. Incidence of autoimmune subepidermal blistering dermatoses in a region of central Germany. Arch Dermatol 1995; 131: 957-8.

5 Murrell DF, Daniel BS, Joly P et al. Definitions and outcome measures for bullous pemphigoid: Recommendations by an international panel of experts. J Am Acad Dermatol 2012; 66: 479-85.

6 Joly P, Roujeau JC, Benichou J et al. A comparison of oral and topical corticosteroids in patients with bullous pemphigoid. N Engl J Med 2002; 346: 321-7.

7 Rzany B, Partscht K, Jung M et al. Risk factors for lethal outcome in patients with bullous pemphigoid: low serum albumin level, high dosage of glucocorticosteroids, and old age. Arch Dermatol 2002; 138: 903-8.

8 Westerhof W. Treatment of bullous pemphigoid with topical clobetasol propionate. J Am Acad Dermatol 1989; 20: 458-61.

9 Wojnarowska F, Kirtschig G, Highet AS et al. Guidelines for the management of bullous pemphigoid. Br J Dermatol 2002; 147: 214-21.

10 Joly P, Roujeau JC, Benichou J et al. A comparison of two regimens of topical corticosteroids in the treatment of patients with bullous pemphigoid: a multicenter randomized study. J Invest Dermatol 2009; 129: 1681-7.

11 Du-Thanh A, Merlet S, Maillard H et al. Combined treatment with low-dose methotrexate and initial short-term superpotent topical steroids in bullous pemphigoid: An open, multicenter, retrospective study. Br J Dermatol 2011; 165: 1337-43.

12 van Velsen SG, Haeck IM, Bruijnzeel-Koomen CA. Percutaneous absorption of potent topical corticosteroids in patients with severe atopic dermatitis. J Am Acad Dermatol 2010; 63: 911-3.

13 van Velsen SG, De Roos MP, Haeck IM et al. The potency of clobetasol propionate: Serum levels of clobetasol propionate and adrenal function during therapy with 0.05% clobetasol propionate in patients with severe atopic dermatitis. J Dermatolog Treat 2012; 23: 16-20.

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General discussion and future perspectives

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General discussion and future perspectives

Fully awareness of new insights of techniques, clinical symptoms and treatment options in pemphigoid diseases is mandatory to be leading in the field. In this thesis we focussed on these three pillars.

In the last decade the incidence in pemphigoid diseases has increased substantially. BP shows a rising incidence two to five times rising to 13.4-21.7 per one million people.[1] Bullous pemphigoid (BP) is a disease of the elderly, and because there is aging of the worldwide population more BP will be diagnosed in the future. However in Finland the age-adjusted incidence of BP was also increased remarkably.[2] The use of multiple drugs in older patients could be an explanation of this rising incidence. Loyd-Lavery et al. reported recently an increased use of loop diuretics in patients with BP before the development of BP, confirming the relation between BP and use of medication.[3] The Netherlands Consortium for Healthy Ageing (NCHA) provides us many different options of research and includes LifeLines which is a scientific study and biobank, where during thirty years 165.000 residents of the Northern Netherlands are being followed. The hypothesis that the rising incidence of BP might be due to the use of multiple drugs needs support by further research such as longterm follow-up in large cohorts as LifeLines.

Direct immunofluorescence (DIF) is because of the high sensitivity the gold standard in the diagnostic approach of pemphigoid diseases. There was still lack of use of the serration pattern analysis by DIF. The image-based online test and instruction video (www.nversusu.umcg.nl) we developed and presented in **chapter 2** was unique and will spread the knowledge of the serration pattern analysis. The test and instruction video will be updated frequently, so participants are triggered to enhance their experience. The test was suitable for every participant, despite the level of experience, and improvement of DIF serration pattern analysis after instruction was shown. Notable was the better recognition of the u-serrated pattern compared with the n-serrated pattern. With the increased knowledge of serration pattern analysis by DIF, more accurate diagnoses can be made in pemphigoid diseases. The actual incidence of epidermolysis bullosa acquisita (EBA) is 0.2-0.5 new case per million people per year.[4] When serration pattern analysis is routinely performed more EBA patients will be diagnosed. Schmidt et al. reported that only 40–60% of EBA serum samples reacted by indirect immunofluorescence microscopy on salt-split skin (SSS) showing dermal binding of the artificial blister, concluding that many EBA patients are underdiagnosed when serration pattern analysis by DIF is lacking.[5] We described that 40% of our EBA serum samples react by indirect immunofluorescence microscopy on salt-split skin (SSS), showing dermal binding. In 45% of our EBA serum samples the NC1/NC2 ELISA is positive. When we combine those two assays we diagnose in 50% of our serum samples EBA.

Without performing DIF serration pattern analysis we miss about 50% of the EBA cases. We demonstrated in 93% of our EBA population the u-serrated pattern by DIF. Another advantage of the serration pattern analysis is the possibility to distinguish the inflammatory EBA from other pemphigoid diseases like BP, mucous membrane pemphigoid (MMP), anti-p200 pemphigoid or linear IgA bullous dermatosis (LAD). Subtyping in pemphigoid diseases is important because of the different prognosis, complications and treatment of the disease. No controlled prospective trials have been reported in the treatment of EBA. In general, treatment is unsatisfactory, especially in the mechanobullous phenotype. A larger EBA population gives the opportunity to conduct randomized controlled trials in the future and treatment in EBA can be optimalized.
Besides DIF, serological analysis was of added value in the diagnostic approach of pemphigoid diseases. Traditionally indirect immunofluorescence (IIF) and IIF on SSS are two valuable assays to detect circulating autoantibodies. Identification of specific autoantigens was not possible with these tests. Immunoblot (IB), enzyme-linked immunosorbent assay (ELISA), immunoprecipitation (IP), knock-out immunofluorescence analysis (KO) and fluorescent overlay antigen mapping (FOAM) are further specifying assays, each with their benefits and disadvantages. Recently commercially available ELISA's for type VII collagen (C7) have emerged, that consists of recombinant NC1 and NC2 C7 domains coated to the plate. In **chapter 3** we confirm the promising use of this ELISA in monitoring disease activity in EBA patients. However the high sensitivity (>93%) and specificity (>96%) described by Saleh et al. and Kim et al. of this ELISA is not based on daily routine, because only SSS positive patients were included in their studies.[6,7] In our study we included SSS positive and SSS negative EBA patients, avoiding selection bias, and show a much lower sensitivity (45%). The use of the C7 ELISA is an investigative contribution, though DIF for serration pattern analysis on skin biopy when suspecting EBA is still mandatory. The C7 ELISA has proven its use in monitoring disease activity in patients.[6,7] This ELISA will be used more frequently in the future for monitoring disease activity when tapering treatment. The anti-C7 ELISA kit containing the recombinant NC1 and NC2 domains (MBL, Nagoya, Japan) would be of great use in IgA EBA patients but does not confirm to current criteria. Monitoring disease activity in this group is desirable and future development of an accurate IgA C7 ELISA might be of great use in a clinical setting.
For the serological diagnosis of pemphigoid diseases multiple techniques like IB, ELISA, IP, KO and FOAM can be used. This is expensive and labor-intensive. Using multiple substrates, including antigen specific substrates, in a single test one can be costs and labor sufficient. Van Beek et al. developed a multiplex IF BIOCHIP mosaic that combined screening and target antigen specific substrates for pemphigus and pemphigoid showing a high sensitivity and specificity.[8]

Zarian et al. used the BIOCHIP in the detection of BP180 autoantibodies in BP patients, describing almost the same diagnostic specificity compared to the NC16A ELISA. The anti-BP230 globular C-terminal domain diagnostic sensitivity was slightly reduced.[9] The multiplex IF BIOCHIP is a new assay which is easy to use, and sensitivity and specificity in current studies are promising. A disadvantage is that it cannot be used as a quantitative measure. Further research and enlargement of the BIOCHIP with envoplakin and rat bladder will give more insights in the specific role of this interesting assay in diagnostics of pemphigoid diseases.

Anti-laminin-332 MMP (Anti-LN-332 MMP) is a rare subtype of pemphigoid that in first instance is difficult to distinguish from other forms of MMP. It is known for the scarring phenotype with airway obstruction due to pharyngeal and laryngeal involvement or lost of vision because of subconjunctival fibrosis and cicatrization.[10] Furthermore patients have an increased relative risk for malignancy, especially adenocarcinoma.[11,12] Because of this clinical aggressive behavior it is important to diagnose patients in an early phase of the disease. Because of new insights presented in **chapter 4** we have described additional major and minor criteria to diagnose anti-LN-332 MMP. The major criteria consists of: (i) subepithelial erosions or blisters on mucous membranes frequently associated with scarring phenotype, (ii) depositions along the BMZ in an n-serrated pattern by DIF, (iii) IgG bound to the dermal side of 1-mol/L NaCL-split human skin by IIF. The minor criteria comprise: (i) anti-LN α3, β3, or γ2 IgG binding by immunoblot analysis on keratinocyte cell extract, (ii) IgG reactivity to native LN-332 by ELISA, (iii) serum immunoprecipitation of LN-332 trimer, (iv) negative IIF on LN-332 deficient skin, while positive IIF on C7 deficient skin, (v) IgG BMZ deposits overlay LN-332 by FOAM. To diagnose anti-LN-332 MMP at least three major criteria, or two major criteria and one minor criterion must be obtained. When these criteria are used adequately, anti-LN-332 MMP will be diagnosed in the early phase of the disease, avoiding serious complications because of doctors delay. Accurate treatment can be introduced and thoroughly screening for malignancies will be performed. An important pitfall is the distinction between anti-LN-332 MMP and anti-p200 pemphigoid. Both diseases show the n-serrated pattern by DIF and dermal binding in SSS. Clinically anti-LN-332 MMP can be distinguished from anti-p200 pemphigoid by dominant mucosal lesions, the oral mucosa and eyes are affected mostly. Anti-p200 pemphigoid presents clinically heterogeneous with tense blisters and urticaria, mimicking BP.[5] According to our new criteria for anti-LN-332 MMP another minor criteria must be obtained when clinical presentation is not according the classical anti-LN-332 MMP phenotype. There is a subset of patients with no confirmative serological test for anti-LN-332 MMP and interestingly with a knock-out analysis showing positive IIF on LN-332 deficient skin, and positive IIF on C7 deficient skin. The use of immunblot or ELISA

showing reactivity with the laminin γ1 epitope could be the answer for the missing link. To date, the pathogenic autoantigen involved in anti-p200 pemphigoid was not revealed. Dainachi et al. performed 2-D gel electrophoresis of dermal extracts and immunoblotting with patients' sera, followed by mass spectrometry analysis of a unique protein band. In 90% of their anti-p200 sera from 32 patients reactivity to the recombinant products of laminin γ1 was seen, suggesting that the C-terminus of laminin γ1 was the autoantigen in anti-p200 pemphigoid. They renamed the disease in anti-laminin γ1 pemphigoid.[13] In 2012 Vafia et al. employed an ex vivo model and showed that autoantibodies in anti-p200 pemphigoid sera are pathogenic while pathogenicity is not mediated by autoantibodies against laminin γ1.[14] The intriguing question is which autoantigen is pathogenic in anti-p200 pemphigoid and how to subtype and name these patients. Further research has to be performed to answer this antigen gap in the pemphigoid diseases spectrum.

In **chapter 5** we described 15 patients with pruritus, immunopathological findings of BP but no blister development. We proposed the unifying term 'pruritic nonbullous pemphigoid', in the spectrum of BP for all patients with immunopathological findings of BP, itch and no blisters, to trigger the dermatologist to think of pemphigoid when confronted with an elderly who complains of itch. We were the first to describe patients (3/15) that only had pruritus without primary skin lesions (pruritus sine materia or 'itch without rash') but immunopathological findings of BP. This observation is of utmost important in the diagnostic algorithm for elderly patients with pruritus in the future. To diagnose 'pruritic nonbullous pemphigoid' preferably DIF must be positive or otherwise SSS positive in combination with another confirmative serological test like Western immunoblot or ELISA. One must be aware that patients with single positive results on NC16A or BP230 ELISA must not be considered as having BP or a BP variant. Feliciani et al. investigated IgG reactivity against the autoantigens of BP, BP180 and BP230, by ELISA in sera of 15 patients with pruritic disorders, without blister development. In 33% of these sera IgG against BP230 and/or BP180 was reported. This confirms the hypothesis that IgG reactivity against BP230, and to a lesser extent against BP180, is a common finding in pruritic disorders of the elderly with a wide clinical spectrum. The relevance of these autoantibodies in patients without clinical symptoms of BP was not cleared. More studies are needed to clarify if these solitary NC16A and BP230 autoantibodies serve as clinical markers in the absence of BP or represent an insignificant epiphenomenon. IgG is the predominant autoantibody in BP patients; however the role of IgE autoantibodies has also been reported before in BP to be pathogenic.[15,16] This insight is of interest for pruritic nonbullous pemphigoid patients. When histological examination of a BP patient is performed a dermal inflammatory infiltrate with eosinophylic spongiosis consisting of

dermal edema associated with eosinophylic infiltration of the upper dermis is frequently seen.[17] Elderly individuals with pruritic dermatosis present often with elevated IgE serum levels. These elevated serum levels were also described in up to 70% of the BP patients. In this same report Dimson et al. showed that in 31 of 32 patients the IgE was bound to the pathogenetic NC16A domain of BP180.[18] This observation led to further research and Zone et al. produced histological blisters of the BMZ in SCID mice after injection subcutaneously an IgE hybridoma to the BP180 ectodomain (LABD97 antigen).[19] Fairley et al. demonstrated that after injecting 6 ng of total IgE isolated from two BP and two normal sera into human skin grafted onto athymic, nude mice revealed the prodromal lesions of BP. After injecting a higher dose of BP IgE (47 ng), histological cleavage of the BMZ was observed in two of the three grafts.[16] These observations suggest a critical role of IgE autoantibodies in the pathogenesis of BP and may be a marker for the intensity of the pruritic symptoms. In the near future sera of our pruritic nonbullous pemphigoid patients will be investigated by IgE NC16A ELISA. To date, only few reports showed IgE depositions along the epidermal basement membrane zone by DIF on patient skin biopsies,[20-22] and future research must be address this issue. Targeted therapy in patients with pruritic BP, high levels of IgE and eosinophils is also of interest. Omaluzimab, a humanized anti-IgE antibody, is used in daily practice in patients with moderate to severe persistent allergic asthma caused by year-round allergens in the air. In vitro experiments have shown that omalizumab is able to induce eosinophil apoptosis and downregulation of pro-inflammatory cytokines synthesized by T- lymphocytes.[23] Recent reports in selected cases show successful treatment with omaluzimab of patients with pruritic BP and elevated IgE levels.[24,25] The efficacy of omaluzimab in patients with BP without high levels of IgE and eosinophils is not known. Future research must be address this issue and might reveal a new promising therapy in pemphigoid diseases. Furthermore, from a pathogenic view, it might be interesting to dissolve the mechanisms behind the absence of blister formation in nonbullous pemphigoid.[26-29] Complement activation has been said to play an important role in the formation of blisters in BP. Therefore, bullous and nonbullous pemphigoid might differ in the way of complement activation. In this respect it is challenging to hypothesize that in nonbullous pemphigoid the IgG antibodies might predominantly be of the IgG4 subclass which has restricted Fc activating abilities and does not activate C1q.[30]
Treatment of BP is challenging because patients are mainly elderly and have comorbidities like neurological and cardiovascular disorders that are associated with the age and the disease.[31] These patients use often multiple drugs and are at high risk of adverse drug reactions and side-effects. In 2010 a Cochrane review was published summarizing the reports for treatment of BP, and concluded that potent topical corticosteroids (CS) are effective and safe, although the

use may be limited because of side effects and practical issues. Systemic oral CS with an initial dose of 0.5-0.75 mg/dag is the best established treatment according to current evidence but iatrogenic morbidity and mortality is reported.[32,33] In **chapter 6** we describe that whole body application of clobetasol propionate cream is effective in the induction phase of treatment in patients with mild BP and in patients with severe BP. The high efficacy of whole body topical clobetasol propionate application is in our opinion due to both local and systemic effects. We confirmed this hypothesis by showing the drop in morning urine cortisol and eosinophil count in patients treated with clobetasol propionate cream on their whole body, sparing the face. Despite of this systemic effect we found in only three patients systemic adverse effects (hypertrichosis, deep vein thrombosis, adrenocortical insufficiency) demonstrating the safety of topical whole body CS. An important question to be asked is with what dose of oral CS the topical potent CS on the whole body therapy can be compared. Van Velsen et al. reported the systemic absorption and effects of whole body application of clobetasol propionate 0.05% cream in patients with atopic dermatitis. Serum concentrations of clobetasol propionate can be measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS).[34] A single skin application resulted in detectable serum levels of clobetasol propionate of 0.112-4.504 ng/ml, and decreased cortisol levels, from 0.47 ± 0.18 µmol/l to 0.04 ± 0.05 µmol/l after 1 day. Moreover the same whole body application of a less potent topical CS (bethamethason valerate) showed in a few patients systemic absorption and fluticasone propionate cream application did not show systemic absorption.[35]

The biological half-life of clobetasol propionate cream is unknown. Hehir et al. reported that cortisol levels remain low until 96 hours after a single application of 25g clobetasol propionate in patients with eczema or psoriasis. Watson et al. showed that cortisol levels were normal 24 hours after a single orally administered dose of 25 mg prednisolone in healthy volunteers. This suggests that the biological half-life of clobetasol propionate cream is longer than the biological half-life of prednisolone. Furthermore this assumes that 20-30 g clobetasol propionate cream is equipotent to 25-40 mg prednisolone by oral administration. Future research must be performed to analyze the systemic effect, the differences and similarities of topical potent CS on the whole body therapy compared to oral CS in the treatment of BP. Rituximab (MabTheraTM; Roche, Basel, Switzerland) is a chimeric human–mouse monoclonal antibody, which binds specifically to the transmembrane antigen CD20 expressed on B-lymphocytes from the pre-B-cell stage to the pre-plasma-cell. Treatment with rituximab shows promising result in patient with pemphigus vulgaris although in pemphigoid diseases literature is limited and rituximab is used frequently as third-line treatment option of recalcitrant BP, MMP, or EBA. Recently Shetty et al. published a

review of the literature of 20 MMP patients who were treated with rituximab using the lymphoma protocol which involves a dose of 375 mg/m^2 administered weekly for 4 consecutive weeks showing that rituximab benefits patient with MMP. Many limitations were addressed in this review, for example the use of concomitant therapy with immunosuppressive and anti-inflammatory agents in 19/20 patients, and long-term follow-up, studies on B-cell levels and antibody responses are lacking.[36] In our Centre for Blistering Diseases we used rituximab in 11 patients with different subtypes of recalcitrant pemphigoid diseases (n=3 BP, n=2 MMP, n=2 EBA mechanobullous phenotype, n=1 OCP, n=1 CP, n=1 IgA-EBA and n=1 LAD). In this group three patients had complete remission (BP, MMP, EBA mechanobullous phenotype), five patients had partial remission (two BP, 1 EBA mechanobullous phenotype, 1 MMP and 1 OCP), and three patients had no response (IgA EBA, LAD and CP). Future research should focus on the efficacy and long-term follow-up of the use of rituximab or other forthcoming anti-CD20 monoclonal antibodies to find their place in the therapeutic armamentarium of pemphigoid diseases.

References

1 Langan SM, Smeeth L, Hubbard R et al. Bullous pemphigoid and pemphigus vulgaris--incidence and mortality in the UK: population based cohort study. BMJ 2008; 337: a180.

2 Forsti A, Jokelainen J, Timonen M et al. Increasing incidence of bullous pemphigoid in Finland. Journal of Investigative Dermatology 2013; 133: s93.

3 Lloyd-Lavery A, Chi CC, Wojnarowska F et al. The associations between bullous pemphigoid and drug use: a UK case-control study. JAMA Dermatol 2013; 149: 58-62.

4 Buijsrogge JJ, Diercks GF, Pas HH et al. The many faces of epidermolysis bullosa acquisita after serration pattern analysis by direct immunofluorescence microscopy. Br J Dermatol 2011; 165: 92-8.

5 Schmidt E, Zillikens D. Pemphigoid diseases. Lancet 2013; 381: 320-32.

6 Saleh MA, Ishii K, Kim YJ et al. Development of NC1 and NC2 domains of type VII collagen ELISA for the diagnosis and analysis of the time course of epidermolysis bullosa acquisita patients. J Dermatol Sci 2011; 62: 169-75.

7 Kim JH, Kim YH, Kim S et al. Serum levels of anti-type VII collagen antibodies detected by enzyme-linked immunosorbent assay in patients with epidermolysis bullosa acquisita are correlated with the severity of skin lesions. J Eur Acad Dermatol Venereol 2012; 27: e224-30.

8 van Beek N, Rentzsch K, Probst C et al. Serological diagnosis of autoimmune bullous skin diseases: prospective comparison of the BIOCHIP mosaic-based indirect immunofluorescence technique with the conventional multi-step single test strategy. Orphanet J Rare Dis 2012; 7: 49.

9 Zarian H, Saponeri A, Michelotto A et al. Biochip technology for the serological diagnosis of bullous pemphigoid. ISRN Dermatol 2012; 2012: 237802.

10 Egan CA, Lazarova Z, Darling TN et al. Anti-epiligrin cicatricial pemphigoid: clinical findings, immunopathogenesis, and significant associations. Medicine (Baltimore) 2003; 82: 177-86.

11 Egan CA, Lazarova Z, Darling TN et al. Anti-epiligrin cicatricial pemphigoid and relative risk for cancer. Lancet 2001; 357: 1850-1.

12 Sadler E, Lazarova Z, Sarasombath P et al. A widening perspective regarding the relationship between anti-epiligrin cicatricial pemphigoid and cancer. J Dermatol Sci 2007; 47: 1-7.

13 Dainichi T, Koga H, Tsuji T et al. From anti-p200 pemphigoid to anti-laminin gamma1 pemphigoid. J Dermatol 2010; 37: 231-8.

14 Vafia K, Groth S, Beckmann T et al. Pathogenicity of autoantibodies in anti-p200 pemphigoid. PLoS One 2012; 7: e41769.

15 Fairley JA, Baum CL, Brandt DS et al. Pathogenicity of IgE in autoimmunity: successful treatment of bullous pemphigoid with omalizumab. J Allergy Clin Immunol 2009; 123: 704-5.

16 Fairley JA, Burnett CT, Fu CL et al. A pathogenic role for IgE in autoimmunity: bullous pemphigoid IgE reproduces the early phase of lesion development in human skin grafted to nu/nu mice. J Invest Dermatol 2007; 127: 2605-11.
17 Fania L, Caldarola G, Muller R et al. IgE recognition of bullous pemphigoid (BP)180 and BP230 in BP patients and elderly individuals with pruritic dermatoses. Clin Immunol 2012; 143: 236-45.
18 Dimson OG, Giudice GJ, Fu CL et al. Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. J Invest Dermatol 2003; 120: 784-8.
19 Zone JJ, Taylor T, Hull C et al. IgE basement membrane zone antibodies induce eosinophil infiltration and histological blisters in engrafted human skin on SCID mice. J Invest Dermatol 2007; 127: 1167-74.
20 Yayli S, Pelivani N, Beltraminelli H et al. Detection of linear IgE deposits in bullous pemphigoid and mucous membrane pemphigoid: a useful clue for diagnosis. Br J Dermatol 2011; 165: 1133-7.
21 Parodi A, Rebora A. Serum IgE antibodies bind to the epidermal side of the basement membrane zone splits in bullous pemphigoid. Br J Dermatol 1992; 126: 526-7.
22 Provost TT, Tomasi TB, Jr. Immunopathology of bullous pemphigoid. Basement membrane deposition of IgE, alternate pathway components and fibrin. Clin Exp Immunol 1974; 18: 193-200.
23 Noga O, Hanf G, Brachmann I et al. Effect of omalizumab treatment on peripheral eosinophil and T-lymphocyte function in patients with allergic asthma. J Allergy Clin Immunol 2006; 117: 1493-9.
24 Dufour C, Souillet AL, Chaneliere C et al. Successful management of severe infant bullous pemphigoid with omalizumab. Br J Dermatol 2012; 166: 1140-2.
25 London VA, Kim GH, Fairley JA et al. Successful treatment of bullous pemphigoid with omalizumab. Arch Dermatol 2012; 148: 1241-3.
26 Bieber K, Sun S, Ishii N et al. Animal models for autoimmune bullous dermatoses. Exp Dermatol 2010; 19: 2-11.
27 Kasperkiewicz M, Zillikens D. The pathophysiology of bullous pemphigoid. Clin Rev Allergy Immunol 2007; 33: 67-77.
28 Liu Z, Giudice GJ, Swartz SJ et al. The role of complement in experimental bullous pemphigoid. J Clin Invest 1995; 95: 1539-44.
29 Nelson KC, Zhao M, Schroeder PR et al. Role of different pathways of the complement cascade in experimental bullous pemphigoid. J Clin Invest 2006; 116: 2892-900.
30 van de Winkel JG, Capel PJ. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. Immunol Today 1993; 14: 215-21.
31 Bastuji-Garin S, Joly P, Lemordant P et al. Risk factors for bullous pemphigoid in the elderly: a prospective case-control study. J Invest Dermatol 2011; 131: 637-43.

32 Kirtschig G, Middleton P, Bennett C et al. Interventions for bullous pemphigoid. Cochrane Database Syst Rev 2010: CD002292.
33 Murrell DF, Daniel BS, Joly P et al. Definitions and outcome measures for bullous pemphigoid: recommendations by an international panel of experts. J Am Acad Dermatol 2012; 66: 479-85.
34 van Velsen SG, De Roos MP, Haeck IM et al. The potency of clobetasol propionate: serum levels of clobetasol propionate and adrenal function during therapy with 0.05% clobetasol propionate in patients with severe atopic dermatitis. J Dermatolog Treat 2012; 23: 16-20.
35 van Velsen SG, Haeck IM, Bruijnzeel-Koomen CA. Percutaneous absorption of potent topical corticosteroids in patients with severe atopic dermatitis. J Am Acad Dermatol 2010; 63: 911-3.
36 Shetty S, Ahmed AR. Critical analysis of the use of rituximab in mucous membrane pemphigoid: a review of the literature. J Am Acad Dermatol 2013; 68: 499-506.

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Summary

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Summary

Pemphigoid diseases is a group of subepidermal autoimmune blistering diseases (sAIBD) characterized by circulating autoantibodies targeting structural proteins that link the cytoskeleton of the basal keratinocytes to the to the underlying epidermal basement membrane zone (BMZ). Binding of the autoantibodies leads to separation of the epidermis and dermis resulting clinically in tense blisters and erosions. Pemphigoid diseases can be divided into different subtypes like bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), ocular cicatricial pemphigoid (OCP), anti-laminin-332 MMP (anti-LN-332 MMP), anti-p200 pemphigoid, anti-plectin pemphigoid, linear IgA dermatosis (LAD), pemphigoid gestationis (PG), lichen planus pemphigoïdes (LPP), Brunsting-Perry pemphigoid and epidermolysis bullosa acquisita (EBA).
The 180-kD antigen (BP180, BPAG2, or type XVII collagen), and the 230-kD antigen (BP230, BPAG1) are the main target antigens in BP, MMP, LAD, PG, OCP, Brunsting-Perry pemphigoid and LPP. LN-332 is the target antigen in anti-LN-332 MMP. EBA patients show circulating autoantibodies targeting collagen VII (coll VII). Anti-p200 pemphigoid is characterized by circulating autoantibodies against the 200 kDa-protein of the lower lamina lucida.
The different subtypes of pemphigoid diseases share clinical characteristics such as tense blisters and erosions although every subtype has its own overall clinical presentation, target antigens, circulating specific autoantibody isotype, treatment and prognosis.
In this thesis we present an overview of pemphigoid diseases with the correlating clinical criteria, optimize the use and interpretation of direct immunofluorescence (DIF), indirect immunofluorescence (IIF), and immunohistochemical assays, and we specify "tailor made" treatment in pemphigoid diseases.
In 2004, our Centre for Blistering Diseases described the serration pattern analysis by routine DIF showing linear n-serration or linear u-serration immunodepositions along the BMZ. The u-serration pattern confirms the diagnosis EBA and represents immunoglobulin depositions in upstanding arms ("grass") of the sublamina densa zone between the rootlets of basal keratinocytes. In all other sAIBDs the antigens are located in the lamina lucida or above, so the immunodeposits follow the rootlets of the basal keratinocytes showing the n-serration pattern. However since its first publication, DIF serration pattern analysis has found limited use, although the criterion is mentioned in textbooks and in the forthcoming European guideline on AIBD. The limited use might be caused by uncertainty and lack of training of the IF microscopists. In **chapter 2** we tested the learnability of DIF serrated pattern recognition under groups with various a priori levels of competence. An online nversusu-test (www.nversusu.umcg.nl) was created, which contained 26 DIF images of BMZ, IgG stained, and photographed with a magnification of 40x

and 63x. All images represented patients with a form of pemphigoid diseases. Thirteen DIF images were presented before and thirteen DIF images after an instruction video about n- and u-serrated patterns. There were three options to choose from: n-serrated, u-serrated or undetermined. The test was completed by three groups of professionals: i) dermatology residents in training at the University Medical Centre Groningen, ii) International experts on bullous diseases, iii) dermatologists and pathologists who participated in the Groningen Blistering Course in the last 10 years. Overall the number of correct answers of serration patterns was significantly higher after instruction than before instruction (median 9.0 correct answers vs. 11.0 correct answers, P<.001). Participants showed a mean improvement after instruction of 15.4% in the UMCG group (66.7% vs. 82.1%), 16.2% in the International expert group (67.2% vs. 83.4%) and 12.1% in the Blistering Course group (60.7% vs. 72.8%). The u-serrated pattern was better recognized than the n-serrated. We concluded that serration pattern analysis by DIF can be learned irrespective of background of expertise.

Recently new ELISA's for the detection of autoantibodies against coll VII have emerged for diagnosing EBA patients. A commercial ELISA is available that has the recombinant NC1 and NC2 coll VII domains coated to the plate, and high sensitivity (>93%) and specificity (>96%) has been reported. A correlation between coll VII ELISA index and disease severity was also demonstrated. All these studies relied on sera that were positive by salt split skin analysis (SSS).

In **chapter 3** we investigated how the type VII coll ELISA would contribute to diagnose EBA in a normal routine setting. We performed type VII coll ELISA on banked sera of 28 EBA patients: 15 SSS-positive and 13 SSS-negative. Sera from healthy blood donors (n=17) and other autoimmune blistering diseases (n=29) served as controls. In four patients ELISA index was measured longitudinally. Serration pattern analysis by DIF was prospectively performed since 2000 and comprised 19 patients. The sensitivity in the SSS-positive group was 80% whereas it was 23% in the SSS-negative group. In the prospective EBA subset it was 45%. The sensitivity of u-serration pattern analysis on skin biopsy was 89%. Ten (53%) of these cases were seronegative by both ELISA and SSS, and would have been missed by serum analysis alone. Of the 46 control sera one serum tested positive (specificity 97.8%). The coll VII ELISA correlated with disease activity over time in individual patients. With these results we show that coll VII ELISA has limited added value in SSS-negative EBA cases. The ELISA test is very valuable in differentiating EBA from anti-laminin-332 MMP and anti-p200 pemphigoid and in its ability to serological monitor EBA patients. We state that u-serration pattern analysis on IF skin biopsy remains the gold standard for the diagnosis of EBA.

Anti-LN-332 MMP is a subtype of pemphigoid diseases characterized by IgG autoantibodies

against LN-332. Anti-LN-332 MMP can clinically resemble other forms of pemphigoid. For diagnosis of anti-LN-332 MMP difficult to obtain laboratory techniques are needed. A correct diagnosis is important because patients with anti-LN-332 MMP have an increased relative risk of malignancy and should be thoroughly oncologically screened. In **chapter 4** we described the clinical features and immunopathological findings in a cohort of 10 Dutch patients. New insights, as described in this chapter, leads to additional criteria to diagnose anti-LN-332 MMP. We presented the following major and minor criteria. Major criteria: 1) Subepithelial erosions or blisters on mucous membranes frequently associated with scarring phenotype, 2) IgG depositions along the BMZ in the n-serrated pattern by DIF, 3) IgG bound to the dermal side of 1-mol/L NaCL-split human skin by IIF. Minor criteria: 1) Anti-LN α3, β3, or γ2 IgG binding by immunoblot analysis on keratinocyte cell extract , 2) IgG reactivity to native LN-332 by ELISA, 3) Serum immunoprecipitation of LN-332 trimer, 4) Negative IIF on LN-332 deficient skin, while positive IIF on type VII collagen deficient skin, 5) IgG BMZ deposits overlay LN-332 by fluorescence overlay antigen mapping (FOAM). To diagnose anti-LN-332 MMP at least three major criteria, or two major criteria and one minor criterion must be obtained. The combination of simple DIF serration pattern and IIF SSS analysis will exclude other forms of MMP and epidermolysis bullosa acquisita from the differential diagnosis.

BP is the most common subtype of pemphigoid diseases and frequently affects elderly and the associated morbidity is significant. In the recent "Definitions and outcome measures for bullous pemphigoid" pruritus, urticaria and tense blisters were reported as the three main clinical pillars of BP. Confusing is the subset of patients with immunopathological findings of BP, pruritus, but no blister development for years. In the literature there is no consensus on how to name this subset of patients. The coined terms include 'pruritic pemphigoid', 'pemphigoid nodularis', 'papular pemphigoid', 'prurigo-nodularis like pemphigoid', 'nonbullous BP', 'prodromal BP', 'cutaneous pemphigoid' and 'BP incipiens'. In **chapter 5** we presented fifteen patients with immunopathological findings of BP who had pruritus sine materia or pruriginous skin lesions without blisters. Diagnosis was reached by a positive DIF with IgG and/or C3c along the epidermal BMZ, or SSS-epidermal binding in combination with a positive ELISA (NC16A or BP230). Clinical symptoms were heterogeneous: pruritus sine materia (no primary skin lesions), eczematous, urticarial, papular and/or nodular skin lesions were seen. Treatment with potent topical corticosteroids or methotrexate sodium (5-15mg/week) led to remission in eleven patients. To trigger the dermatologist to think of pemphigoid when confronted with an elderly who complains of itch we proposed the unifying term 'pruritic nonbullous pemphigoid'. We state that when an elderly with therapeutic refractory itch is presented in the clinic, DIF and IIF must be

performed to exclude pruritic nonbullous pemphigoid.
Treatment of BP is challenging because of the older age of the patients, and the comorbidities like neurological and cardiovascular disorders that are associated with the age and the disease. Current standard of treatment of BP is systemic oral corticosteroids (CS). However significant iatrogenic morbidity and mortality is reported. Studies have shown that topical potent CS is safer than oral prednisolone in BP. In **chapter 6** we examined the efficacy and adverse effects of whole body topical clobetasol propionate cream application in patients with mild or severe BP. Moreover we investigated cortisol levels and eosinophil counts in selected cases and found systemic effects using a potent topical CS. We performed a retrospective analysis of a series of mild (n=40) and severe (n=34) BP patients, treated with ranging doses (20-40g/day) clobetasol propionate cream. For assessing systemic effects we observed in selected cases eosinophil count and morning urine cortisol level. Patients with mild BP achieved in 90.0% disease control and in severe BP in 73.5%. Complete remission was achieved in mild BP in 64.1% (35.9% off therapy and 28.2% on therapy) versus 41.2% in severe BP (5.9% off therapy and 35.3% on therapy). Local adverse effects were mainly skin atrophy (14.9%) and purpura (5.4%). Systemic adverse effects were rare (n=3) and consisted of deep vein thrombosis, hypertrichosis and adrenocortical insufficiency. Systemic effect was witnessed by immediate drop of eosinophil count, and decrease in the morning urine cortisol in selected cases. These results show that whole body application of clobetasol propionate cream is effective in the induction phase of treatment in patients with mild BP and in patients with severe BP. We advice to add an immunosuppressive drug in BP patients with a relapse when topical clobetasol propionate cream is not sufficient anymore. The whole body clobetasol therapy is safe and may be better tolerated than systemic oral CS.

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Samenvatting

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Samenvatting

Pemfigoïd is een heterogene groep subepidermale autoimmuun blaarziekten (sAIBD) gekenmerkt door circulerende autoantistoffen gericht tegen eiwitten in de epidermale basaalmembraan zone (BMZ). De epitheelcellen van de huid zijn via de hemidesmosomen (HD's) verbonden met de onderliggende dermale matrix en defecten in deze adhesiecomplexen zorgen voor fragiliteit en blaarvorming van de huid en slijmvliezen. In het het pemfigoïd spectrum zijn vele subtypen te onderscheiden zoals: bulleus pemfigoïd (BP), slijmvlies pemfigoïd/mucosaal membraneus pemfigoïd (MMP), oculair cicatricieel pemfigoïd (OCP), anti-laminine-332 slijmvlies pemfigoïd (anti-LN-332 MMP), anti-p200 pemfigoïd, anti-plectine pemfigoïd, lineaire IgA dermatose (LAD), pemfigoïd gestationis (PG), lichen planus pemphigoïdes (LPP), Brunsting-Perry pemfigoïd en epidermolysis bullosa acquisita (EBA). Het 180-kD antigeen (BP180, BPAG2 of type XVII collageen) en het 230-kD antigen (BP230, BPAG1) zijn de belangrijkste target antigenen bij BP, MMP, LAD, PG, OCP, Brunsting-Perry pemfigoïd en LPP. Bij anti-LN-332 MMP zijn de circulerende autoantilichamen gericht tegen LN-332 en bij EBA tegen collageen VII (coll VII). Anti-p200 pemfigoïd is een zeldzame vorm van pemfigoïd waarbij autoantistoffen tegen het 200-kDa eiwit van de lamina lucida worden aangetoond. Er zijn enkele klinische overeenkomsten tussen de verschillende subtypen zoals pral gespannen blaren, erosies en, zoals in tegenstelling tot bij pemphigus, een negatief teken van Nikolsky. Echter er zijn ook unieke verschillen qua klinische symptomen, target antigenen, antilichaam subtype, behandeling en prognose te definiëren.

In dit proefschrift wordt een overzicht gegeven van het pemfigoïd spectrum met de daarbij behorende klinische symptomen, het optimale gebruik en interpretatie van diagnostiek zoals directe immunofluorescentie (DIF), indirecte immunofluorescentie (IIF) en detectie van antigeen specifieke circulerende autoantilichamen in het serum én het toepassen van een "tailor made" behandeling bij de pemfigoïd patiënt.

In 2004 is in ons blaarexpertise centrum te Groningen de serratiepatroon analyse van de DIF beschreven waarbij een n-serratie of een u-serratie patroon van de immunodeposities langs de BMZ kan worden aangetoond. Lineaire deposities vanaf de lamina densa en hoger hebben een n-geserreerd patroon, terwijl deposities onder de lamina densa een u-serratie patroon vertonen. Het u-serratie patroon ziet men alleen bij de circulerende autoantistoffen tegen coll VII, zoals bij EBA of bulleuze systemische lupus erythematosus en het n-serratie patroon bij de overige vormen van het pemfigoïd spectrum. Tot op heden wordt er in de dagelijkse praktijk, behoudens in ons blaarexpertise centrum, de serratiepatroon analyse van de DIF nog te weinig toegepast. De minimale toepassing wereldwijd zou te maken kunnen hebben met onzekerheid

in de beoordeling van het serratiepatroon of het ontbreken van expertise en training van dermatologen en pathologen. In **hoofdstuk 2** wordt een studie beschreven waarin de leerbaarheid van de interpretatie van de DIF serratiepatroon analyse wordt getest bij groepen met verschillend niveau van expertise in het pemfigoïd spectrum. Een online nversus-test (www.nversus.umcg.nl) is ontwikkeld, die 26 DIF afbeeldingen van de BMZ bevat, IgG gekleurd, en gefotografeerd met een 40x en 63x vergroting. Alle afbeeldingen zijn van patiënten met een vorm van pemfigoïd. Dertien DIF afbeeldingen werden getoond voor een instructievideo en dertien DIF afbeeldingen werden getoond na een instructievideo over de beoordeling van DIF serratiepatroon analyse. De deelnemers hadden drie keuze opties: n-serratie, u-serratie of niet te bepalen. De test werd verricht door drie verschillende groepen deelnemers: i) dermatologen en dermatologen in opleiding (i.o.) van het Universitair Medisch Centrum Groningen (UMCG), ii) Internationale experts op het gebied van auto-immuun blaarziekten (AIBD), iii) dermatologen en pathologen die deelnamen aan de Groninger Blaarcursus tussen 2002-2012. Het aantal correcte antwoorden van het serratiepatroon was significant hoger na instructie dan voor de instructie (mediaan 9.0 correcte antwoorden vs. 11.0 correcte antwoorden, P<.001). Deelnemers aan de test toonden een gemiddelde verbetering na instructie van 15.4% in de UMCG groep (66.7% vs. 82.1%), 16.2% in de Internationale expert groep (67.2% vs. 83.4%) en 12.1% in de Blaarcursus groep (60.7% vs. 72.8%). Het u-serratie patroon werd beter herkend dan het n-serratie patroon. Onze conclusie was dat DIF serratiepatroon analyse geleerd kan worden onafhankelijk van het expertiseniveau van de beoordelaar.

Recent zijn er nieuwe ELISA's ontwikkeld voor de detectie van autoantistoffen tegen coll VII, die kunnen worden toegepast om EBA te diagnosticeren. Er is een commerciële ELISA beschikbaar waarbij zowel het NC1 als het NC2 domein op de plaat is gecoat. In de literatuur is van deze ELISA een hoge sensitiviteit (>93%) en specificiteit (>96%) beschreven. Tevens is er een correlatie aangetoond tussen de ernst van de EBA bij patiënten en de hoogte van de ELISA index. Al deze studies zijn gebaseerd op EBA patiënten met een positieve zoutgespleten humane huid (SSS). In **hoofdstuk 3** hebben we onderzocht hoe de bijdrage is van de type VII coll ELISA in de diagnostiek bij EBA patiënten in de normale dagelijkse praktijk. We verrichten de type VII coll ELISA op sera van 28 EBA patiënten uit onze databank: 15 patiënten met een positieve SSS en 13 patiënten met een negatieve SSS. Serum van gezonde bloeddonoren (n=17) en van patiënten met een andere vorm van sAIBD (n=29) dienden ter controle. Bij vier patiënten werd de ELISA index gedurende het ziektebeloop gemeten. DIF serratiepatroon analyse werd prospectief uitgevoerd vanaf 2000 (n=19). De sensitiviteit in de positieve SSS groep was 80%, en in de negatieve SSS groep 23%. In de prospectieve EBA populatie toonden we een sensitiviteit van slechts 45%

aan. De sensitiviteit van DIF u-serratie patroon analyse was 89%. Tien van deze patiënten waren seronegatief bij zowel de ELISA als de SSS, en zouden dus niet als EBA gediagnosticeerd worden als alleen serum geanalyseerd zou worden. Van de 46 controle sera, was er één positieve ELISA (specificiteit 97.8%). De coll VII ELISA index correleerde met het ziektebeloop bij individuele EBA patiënten. Met deze resultaten toonden wij aan dat de coll VII ELISA van weinig toegevoegde waarde is in SSS negatieve EBA patiënten. De meerwaarde van de coll VII ELISA is met name dat het EBA kan onderscheiden van anti-LN-332 MMP en anti-p200 pemfigoïd patiënten en tevens dat het de ziekteactiviteit bij EBA kan monitoren. Echter de DIF u-serratiepatroon analyse blijft de gouden standaard bij de diagnostiek voor EBA.

Anti-LN-332 MMP is een zeldzame vorm van pemfigoïd waarbij de IgG autoantilichamen gericht zijn tegen LN-332. Klinisch kan anti-LN-332 MMP lijken op andere vormen van pemfigoïd. Voor het stellen van de diagnose anti-LN-332 MMP zijn moeilijk verkrijgbare laboratorium technieken nodig. Een correcte diagnose is noodzakelijk omdat patiënten met anti-LN-332 MMP een relatief verhoogd risico op maligniteiten hebben en daarom ook ten tijde van de diagnose oncologisch gescreend moeten worden. In **hoofdstuk 4** hebben wij de klinische symptomen en immunopathologische bevindingen van tien patiënten uit ons blaarexpertise centrum beschreven. Nieuwe inzichten, zoals benoemd in dit hoofdstuk, hebben geleid tot additionele criteria voor de diagnose anti-LN-332 MMP. We hebben een onderverdeling gemaakt in "major" en "minor" criteria. Major criteria: 1) Subepidermale erosies of blaren op slijmvliezen met frequent verlittekening, 2) IgG immunodeposities langs de BMZ in het n-serratie patroon, 3) IgG in de bodem van de blaar bij SSS. Minor criteria: 1) IgG binding aan de $\alpha 3$, $\beta 3$, of $\gamma 2$ keten van LN-332 bij immunoblot, 2) positieve LN-332 ELISA, 3) Immunoprecipitatie positief voor LN-332, 4) Negatieve IIF op LN-332 deficiënte huid, terwijl positieve IIF op coll VII deficiënte huid, 5) IgG BMZ deposities bedekken LN-332 bij fluorescence overlay antigen mapping (FOAM). Voor de diagnose anti-LN-332 MMP moeten er tenminste drie major criteria, of twee major en één minor criterium behaald worden. De combinatie van DIF serratiepatroon analyse en SSS zal andere vormen van pemfigoïd en EBA uitsluiten van de differentiaal diagnose.

BP is het meest voorkomende subtype van het pemfigoïd spectrum en wordt vaak bij ouderen gediagnosticeerd en is geassocieerd met een significante morbiditeit. In de recent verschenen "Definitions and outcome measures for bullous pemphigoid" worden jeuk, urticaria en pral gespannen blaren genoemd als de drie belangrijkste kenmerken van BP. Verwarrend is de subgroep patiënten met immunopathologische bevindingen passende bij BP, jeuk en geen ontwikkeling van blaren voor jaren. In de literatuur is er geen consensus hoe deze subgroep te definiëren. Vooralsnog worden er verschillende definities gebruikt zoals 'pruritic pemphigoid', 'pemphigoid

nodularis', 'papular pemphigoid', 'prurigo-nodularis like pemphigoid', 'nonbullous BP', 'prodromal BP', 'cutaneous pemphigoid' and 'BP incipiens'. In **hoofdstuk 5** presenteerden wij vijftien patiënten met immunopathologische bevindingen passende bij BP, met daarbij pruritis sine materia of jeukende huidafwijkingen zonder blaren. De diagnose werd gesteld op basis van een positieve DIF met IgG en/of C3c langs de BMZ, of SSS epidermale binding van IgG in combinatie met een positieve ELISA (NC16A of BP230). De klinische symptomen waren heterogeen en bestonden uit: pruritus sine materia (geen primaire efflorescenties), eczemateuze, urticariele, papuleuze en/of nodulaire huidafwijkingen. Behandeling met potente topicale corticosteroïden (CS) of methotrexaat (5-15 mg/week) gaf complete remissie in elf patiënten. Om de dermatoloog te prikkelen om aan pemfigoïd te denken bij ouderen met jeuk stelden wij de allesomvattende definitie voor: 'pruritic nonbullous pemphigoid'. Wij stelden dat wanneer een oudere patiënt met therapeutisch refractaire jeuk zich presenteert op de polikliniek, DIF en IIF verricht moeten worden om de diagnose 'pruritic nonbullous pemphigoid' aan te tonen of uit te sluiten.
Behandeling van BP is een hele uitdaging gezien de oudere leeftijd van de patiënt en de comorbiditeiten zoals neurologische en cardiovasculaire ziektes die zijn geassocieerd met de leeftijd en met BP. De huidige aanbevolen behandeling bestaat uit systemische CS, echter hoge significante morbiditeit en mortaliteit is beschreven.
Studies hebben beschreven dat topicale potente CS veiliger zijn dan behandeling met orale CS voor BP. In **hoofdstuk 6** onderzochten we de effectiviteit en bijwerkingen van de applicatie van clobetasol propionate crème van kaak tot teen bij patiënten met milde of ernstige BP. Verder analyseerden wij de ochtend urine cortisol spiegel en eosinofiele granulocyten in een aantal geselecteerde patiënten. In een retrospectieve analyse van een serie milde (n=40) en ernstige (n=34) BP patiënten, werden deze patiënten behandeld met clobetasol propionate crème (dosis 20-40g/dag) van kaak tot teen. Patiënten met milde BP bereikten in 90% ziekte controle en in ernstige BP in 73.5%. Complete remissie werd in 64.1% (35.9% zonder therapie en 28.2% met therapie) bereikt bij milde BP versus 41.2% (5.9% zonder therapie en 35.3% met therapie) bij ernstige BP. Lokale bijwerkingen bestonden vooral uit huidatrofie (14.9%) en purpura (5.4%). Systemische bijwerkingen waren schaars (n=3) en waren een diep veneuze trombose, hypertrichosis en bijnierschorsinsufficiëntie. Systemische effecten van de topicale behandeling met clobetasol propionate crème van kaak tot teen werden geobjectiveerd door daling van de perifere eosinofielen, en een daling in het ochtend urine cortisol. Deze resultaten toonden dat clobetasol propionate crème van kaak tot teen een effectieve en veilige behandeling is in de inductie fase van de behandeling van patiënten met milde en ernstige BP. Clobetasol propionate crème van kaak tot teen wordt beter verdragen dan systemische CS door de patiënt. We adviseren om een

immuunsuppresivum toe te voegen als steroïdsparend adjuvans wanneer er een recidief optreedt en topicale behandeling niet meer afdoende is.

8

A

Appendices

List of publications
Dankwoord
Curriculum Vitae

Centre for Blistering Diseases, Department of Dermatology,
University of Groningen, University Medical Centre Groningen,
Groningen, the Netherlands

List of publications

de Groot AC, Terra JB, Jonkman MF.
Hoe schrijf je een wetenschappelijk artikel.
Ned Tijdschr Dermatol Venereol 2007; 17: 394-401

Terra JB, Dikkers FG, Snelleman JA, Jonkman MF.
Anti-laminine 332 slijmvliespemfigoïd.
Ned Tijdschr Dermatol Venereol 2008; 18: 339-341

Terra JB, Jonkman MF.
Een zwangere vrouw met blaren.
Ned Tijdschr Geneesk 2009;153: B36

Terra JB, Pas HH, Hertl M, Dikkers FG, Kamminga N, Jonkman MF.
Immunofluorescence serration pattern analysis as a diagnostic criterion in anti-laminin-332 mucous membrane pemphigoid-immunopathological findings and clinical experience in 10 Dutch patients-.
Br J Dermatol 2011; 165(4):815-22

Brandt O, Rafei D, Podstawa E, Niedermeier A, Jonkman MF, Terra JB, Hein R, Hertl M, Pas HH, Müller R.
Differential IgG recognition of desmoglein 3 by paraneoplastic pemphigus and pemphigus vulgaris sera.
J Invest Dermatol 2012;132(6):1738-41

Blok JL, Reesink-Peters N, Diercks GF, Reyners AK, Terra JB.
Vulvar basall cell carcinoma with destructive consequences.
Ned Tijdschr Geneeskd 2012;156(40):A5391

Terra JB, Jonkman MF, Diercks GF, Pas HH.
Low sensitivity of type VII collagen ELISA in epidermolysis bullosa acquisita: serration pattern analysis on skin biopsy is required for diagnosis.
Br J Dermatol 2013;169(1):164-7.

Terra JB, Meijer JM, Jonkman MF, Diercks GF.
The n-vs. u-serration is a learnable criterion to differentiate pemphigoid from epidermolysis bullosa acquisita in direct immunofluorescence serration pattern analysis
Br J Dermatol 2013; 169(1):100-5

Bakker CV, Terra JB, Pas HH, Jonkman MF.
Bullous pemphigoid as pruritus in the elderly: a common presentation.
JAMA Dermatology 2013; 149(8): 950-3

Terra JB, Potze WJ, Jonkman MF.
Whole body application of a potent topical corticosteroid for bullous pemphigoid.
J Eur Acad Dermatol Venereol. 2013 April (Epub ahead of print)

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Curriculum Vitae

Jorrit Bastiaan Terra werd geboren op 27 september 1978 te Amsterdam. In 1996 behaalde hij het Atheneum B diploma aan het Casimir Lyceum te Amstelveen. Nadien heeft hij gedurende één jaar Medische Biologie gestudeerd aan de Vrije Universiteit te Amsterdam.
In 1997 was de numerus fixus hem gunstiger gezind en volgde hij de studie Geneeskunde aan de Vrije Universiteit te Amsterdam en behaalde Cum laude het artsexamen in 2004. Na het behaalde artsexamen heeft hij gedurende één jaar gewerkt als arts-assistent niet in opleiding bij de afdeling Heelkunde van het Spaarne ziekenhuis te Hoofddorp. In 2005 begon hij met de opleiding Dermatologie aan het Universitair Medisch Centrum Groningen met als opleider prof. dr. M.F. Jonkman. In oktober 2010 werd de opleiding tot dermatoloog afgerond en trad hij toe tot de staf van de afdeling Dermatologie van het Universitair Medisch Centrum Groningen en werd zijn promotietraject gestart. Zijn aandachtsgebieden zijn immunodermatologie, dermato-oncologie en dermatochirurgie.